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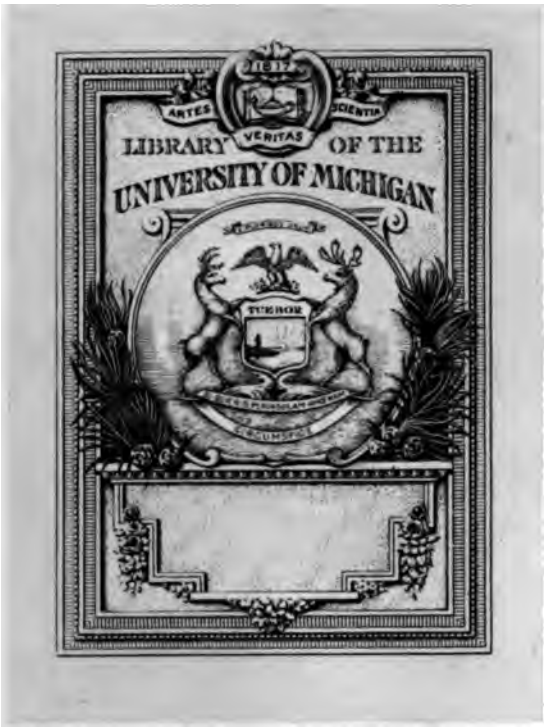
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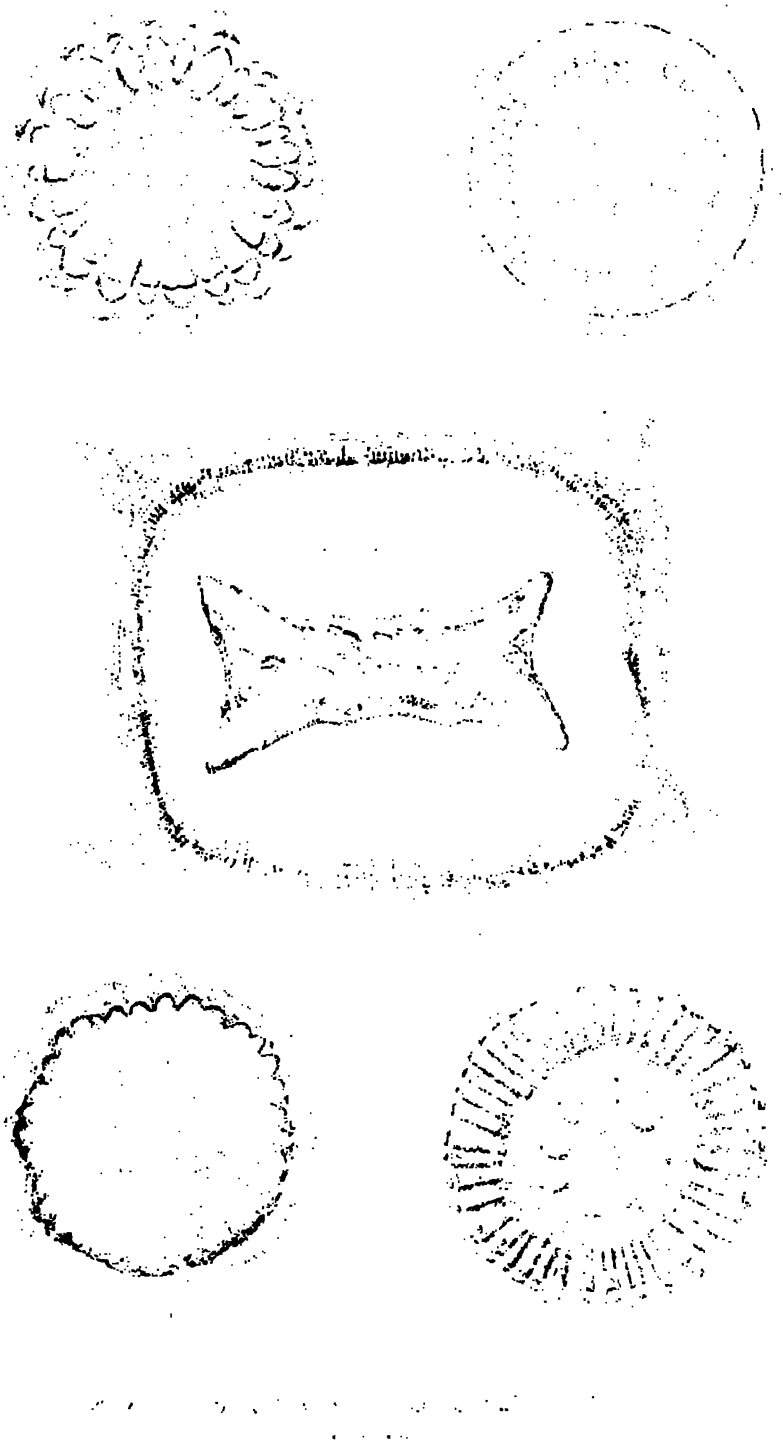

PRACTICAL MICROSCOPY.

BY

GEORGE E. DAVIS,

F.R.M.S., F.I.C.,

ETC., ETC.

ILLUSTRATED WITH THREE HUNDRED AND TEN ILLUSTRATIONS AND A COLOURED FRONTISPIECE.

NEW AND REVISED EDITION.

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1889.

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PREFACE TO THE NEW EDITION.

THE call for a new edition of *Practical Microscopy* has given an opportunity for a complete revision of the work. The volume now presented to the reader has been enlarged and brought as nearly as possible down to the present time; and not only has this been done with regard to English instruments, but the scope of the work has been extended so as to include the apparatus in general use upon the Continent and also in the United States of America.

The Americans have, especially in later years, made some very decided improvements in the construction of the microscope; mechanical and optical improvements, and all those deemed worthy of special notice have been included in the pages which follow. Since the Second Edition of this work was published the renowned constructor of objectives, TOLLES, has passed to his long rest, and his champion, Charles Stodder, has also followed him; but Tolles has left monuments behind him, built with his own hands, in the

form of some of the best objectives the world has ever seen.

But while objectives of the widest apertures have been constructed here, on the Continent, and in America, of the old, or let us say, the usual optical glass, a new direction has been mapped out in Germany by the introduction of apochromatics. A new glass has been the means of reducing the secondary spectrum to a minimum, but whether such glass is capable of wearing well and of resisting the action of the air is as yet non-proven. Many readers will be aware of our own chemist's (FARADAY) researches into the subject of optical glass of high density and its failure to resist the process of time.

The Author's theme has been to make this work essentially a practical one. As little theory as is consistent with a proper understanding of the subject-matter has been introduced; but it was thought desirable to give a short popular exposition of the leading features of the aperture question, and no better course seemed possible to the author than to give almost verbatim the paper read by Mr. W. Blackburn before the Manchester Microscopical Society a few years ago.

The chapter on the Delineation of Objects has been added to, chiefly in the direction of enabling students to keep a correct record of their observations, as well as to enable the readers of papers before learned societies to illustrate them, either for the evening, or for publication. It is to be hoped the information given herein will prove a stimulus in this direction.

Many of the illustrations found herein have been prepared expressly by the Author, by methods described in the thirteenth chapter, so that their practicability is hereby established and needs no further demonstration.

As far as the preparation and mounting of objects is concerned the Author is well aware that much more might have been written on this subject, and it is mainly in this direction that future extensions must be made. But the principle has been recognized that all operations in microscopy must be carried out with intelligence, and the why and wherefore of each well understood, so that instead of multiplying instances of mounting operations, certain types have been selected, which the student should follow out carefully, and only depart from as his knowledge becomes more extensive.

The aim all through the work has been to furnish such information as would enable the microscopist to thoroughly understand the instrument and the principles upon which it is constructed, and to initiate him into the art and mystery of those operations which go hand in hand with scientific enquiry, and with which the use of the microscope is intimately connected.

The Microscope is a civilizing instrument; it enlarges and expands the mind of man, and brings prominently forward the best side of human nature; let us foster and encourage it amongst us.

The Author desires to thank all those correspondents, English, Continental, and American, who so kindly favoured him with criticisms of the First and Second Editions, and he

hopes that the result of those communications has been to make the new edition still more valuable to the worker with the microscope, and to establish it as a permanent text-book.

GEORGE E. DAVIS.

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PRACTICAL MICROSCOPY.

CHAPTER I.

INTRODUCTION.

DURING the past few years rapid strides have been made in the manufacture and supply of cheap and at the same time really good working microscopes; and these having been extensively purchased, have extended and disseminated a taste for the study of minute things, in those to whom the possession of a microscope a few years ago seemed a luxury to be only dreamt of.

Good works treating upon the practical use of the microscope and its accessories, have hitherto been of so expensive a character as to be certainly beyond the reach of most students, and many who might have been led into the paths of scientific research, have probably kept their instruments to gratify their curiosity or that of their friends. Such persons often think that the possession of a microscope must always make a microscopist, and in order to study objects, that it is only necessary to place them on the stage of the microscope when their hidden structure will manifest itself at once. No greater mistake could ever be made. It is very desirable that the student should be thoroughly acquainted with the physics and chemistry of the science, for when once these are mastered he will see his way to carry on

experiments in a rational manner, as often as fresh problems are presented to him.

Again, no inexpensive work upon practical microscopy brings the subject down to the present time; this and other minor details urged us to cut out a path for ourselves, and produce a treatise which might be of use to the student.

In using the microscope as a means of gaining an insight into the anatomy or life-history of forms only faintly distinguished by the unassisted eye, the student must be prepared to exercise a certain amount of patience; skill in manipulation comes by practice only, and before commencing with any investigation, all preconceived notions should be cast aside, the observer being guided chiefly by the results of his own observations.

Isolated observations should never be considered conclusive; experiments require frequent repetition under very varying conditions before we can accept the results as true interpretations of what we have actually seen. We should also be careful to take into account the degree of perfection of our instruments as well as our own capability of vision; what one observer will see, another will fail to detect, and if any preconceived notions exist, the observations are sure to be moulded after the same fashion.

Expertness in microscopy is only to be attained by study and practice, and as it is necessary, in order to progress, that we take up the subject where our predecessors left off, it is necessary to become acquainted with what has been done previously, and this may be arrived at by the perusal of old standard works upon the science, such as those of Baker, Pritchard, and Goring, or the more recent work of Mr. Quekett's, "The Practical Treatise on the Use of the Microscope." These works, though old, are valuable, and the executive of every microscopical society should endeavour to secure a copy of each for its library.

If microscopical students would only peruse these older works, and enter into communication and discussion with their brother microscopists, much time and work would be saved, and a great deal of that *re-discovery* which goes on at the present day averted; there is no other way of avoiding repetitions except by the methods above stated.

We might advance more rapidly, or more certainly at least, if we asked, before performing any microscopical operation, the reason why. We are too apt to do things in a certain manner because someone who instructed us in the art and mystery did so before us; no inquiry was made, but we did likewise. If the principles upon which all operations are conducted were thoroughly understood by all those who intend working with the microscope, much labour would be saved, needless experiments avoided, slides rendered more permanent, and microscopical research brought more into favour on account of some of the barriers which now obstruct progress having been broken down.

It is in this direction that a treatise may be useful to the student; objects themselves, whether of animal, vegetable, or mineral origin, are best treated of in works entirely independent of practical microscopy; an organism, however minute, has its life-history, and it should be the aim of each student to be useful in his generation by endeavouring to furnish an accurate account of the cycle of existence of some member of the animal or the vegetable world.

Of course, before he can be expected to occupy himself with original research, the microscopist should be fairly acquainted with all that has been done before upon the subject; but above all must he be familiar with the instrument with which he works, and the methods whereby certain results may be attained.

Microscopical research does not always require the aid of expensive apparatus. It is very handy and often saves time,

no doubt, to have ready all those accessories so ingeniously devised by the makers, for microscopists with long purses; but apparatus to answer the same purposes may often be made by the ingenious worker, which, though not possessing such a good appearance, serve just as well as the more expensive articles.

Many microscopists, after a few years' devotion to their favourite instrument, find themselves encumbered with a host of paraphernalia of no use to themselves or to anyone else, the cost of which might have been saved by considering beforehand the capabilities of the required apparatus. As to instruments, each individual taste has to be considered, some prefer one pattern and some another; but, after all, these matters are easily arranged if the principle of construction is good.

The main office of the microscope is that of enlargement; but this amplification of the image of an object must be attained without distortion or the introduction of colours not in the original, and it is because single lenses give images blurred with spherical and chromatic aberration that double and triple combinations are used in the construction of all good microscopes. Single lenses are however very useful for general purposes: as a pocket lens, it prevents the collection of much useless material during a naturalist's rambles, and upon reaching home a further use is found in its employment as an aid in dissecting or in mounting the objects culled. The most useful magnifiers are the ordinary watchmakers' or engravers' eye-glasses in the usual horn mount, but their amplifying power should not be too great or continued use may impair the eyesight.

A combination of several single lenses, such as is shown in Fig. 1, is much used as a pocket magnifier for the field where a high power is not required; they are cheap, but the lower powers only are generally useful. The Stanhope and

Coddington lenses are also used by some collectors, though there is little doubt that they are going out of date on account of their not being so useful as newer forms of the simple microscope.

The Stanhope magnifier is a double convex lens having two unequal curvatures. In observing, the deepest curve is placed towards the eye, the object adhering to the least convex side being just in focus. The Coddington lens is generally a sphere of glass, round the periphery of which a deep groove has been cut and filled up again with black cement. This lens focusses at a short distance from the object, and is much superior to the Stanhope form. Inferior Coddingtons are now made from rejected double convex lenses which do not act as well as the form described.

FIG. 1.

Without doubt the best magnifiers for field use and such work generally are the platyscopic lenses of Mr. John Browning, which he makes of three degrees of power, amplifying 15, 20, and 30 diameters respectively. They are really achromatic triplets, are set in ebonite cells, and mounted in tortoise-shell frames. These lenses focus at about three times the distance from an object as a Coddington of the same power, and so allow of the easy examination of opaque objects. They are shown engraved full size in Fig. 2. Steinheil has produced similar lenses, which he terms "aplanatische loupen"; they are of similar construction to the above, and are made to magnify 5½, 8, 12, 16 and 24 diameters.

These lenses were not much known in England until after 1872, the introduction of them being due to Professor Markoe, who in that year exhibited them to several

microscopists in London. Miller, of New York, who used to work for Tolles, makes the same kind of lens.

One of these lenses, or, preferably, two of them, carried in the pocket when field hunting, will prove of invaluable assistance to the student. The most useful powers will be found in those amplifying 15 and 30 diameters: the former serves well for the examination of mosses, ferns, lichens, algæ, and such members of the animal world as can be

Fig. 2.

recognised by the aid of a 3-inch objective and the A eye-piece, to which combined, it is about equal in power; whilst the smaller glass, giving greater amplification, answers admirably for micro-fungi, minute algæ and lichens, and those forms of animal life for which a 1½-inch objective is desirable.

If the reader refers to any work treating upon optics, he will find that convex lenses yield images in two distinct manners—*virtual* images and *real* images. A double convex lens, when used as an ordinary magnifying glass, produces a

virtual image which is erect and larger than the object, a may be seen by reference to Fig. 3.

The greater the convexity given to the surfaces of the lens, the more will it amplify, and it may also be said that

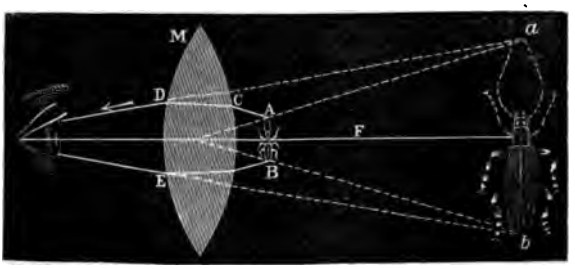


Fig. 3.

the nearer the object be placed to the principal focus of the lens (F) for parallel rays, the larger will the virtual image appear. To yield a virtual image, the object must be placed between the lens and its principal focus.

A *real* image is formed from a double convex lens, only when the object is outside, or in front of, the principal focus for parallel rays; this image is inverted, and may be studied by receiving it upon a screen of ground glass. The action

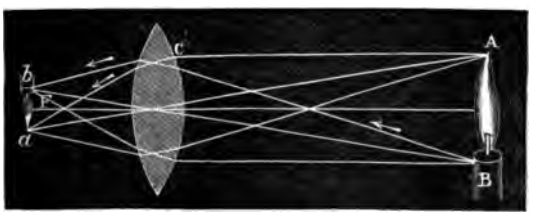


Fig. 4.

of lenses, in the production of real images, should be carefully considered by the student, as upon it depends chiefly the results of his manipulation, which may be perfect or defective in proportion as he understands the theory of this simple optical instrument.

An inspection of Fig. 4 will show the two consequences of this action. If A B represents a lighted candle placed far distant from the principal focus of the lens, a small, real but inverted image of the candle will be thrown upon the screen at *a b*, and the further A B is placed away from the principal focus for parallel rays, the smaller will be *a b*, and the nearer will it be formed to the opposite principal focus. On the other hand, if *a b* represents the lighted candle placed in front of the principal focus F, but very near to it, the real image which is formed, A B, is much amplified, in proportion as *a b* is nearer to the principal focus, and the distance at which A B is formed is likewise regulated by the distance of the object from this point. The nearer *a b* is to F, the further will A B be formed away from the lens, and *vice versâ*.

The student will now be able to see the principle upon which the microscope is constructed, the objective producing a real image of the object, while a virtual image of this enlargement is seen by means of the eye-piece; and in closing this chapter, we do so by giving a table of the magnifying power of single convex lenses of varying focal lengths, for parallel rays at a distance of 12½ inches from the micrometer to the screen, which has been constructed from a paper upon the subject by Dr. Woodward in 'Silliman's Journal' for June, 1872.

Focal Distance for Parallel Rays.	Amplification in Diameters.
2″	4·0
1″	10·4
½″	23·0
¼″	48·0
⅛″	98·0
1/16″	198·0

CHAPTER II.

THE MICROSCOPE STAND.

AS we are supposed to be treating nearly exclusively of compound microscopes—that is to say, of instruments in which the amplification of an object is produced by means of a combination of lenses called an "objective," the image being further magnified by another set called an "ocular" or "eye-piece"—we will consider that our readers require no demonstration as to the necessity of some mechanical contrivance for holding these lenses in their correct positions.

This is the office of the stand of a compound microscope, and when we notice the progress which has been made in the details of this instrument from the time the first was made for sale in England by Mr. John Marshall, we shall find that not to opticians only, properly so called, are we indebted for improvements, but to mechanicians equally. It was but of little use attempting to perfect the optical arrangements while the mechanical contrivances were imperfect, and when this was fully appreciated, real improvements were made by the mechanic and opticians working hand in hand. At the same time, we must not forget the aid which has been rendered by amateurs and microscopists with unlimited means at their disposal; it is certain that most of the early, and many even of the more recent improvements would not have been executed, except at the instance of those possessing well-lined purses and with great interest for the science.

As previously mentioned, the chief function of the microscope stand is to receive the eye-piece and objectives;

Fig. 5.

but it has to do much more than this, and upon its form and general construction, together with the character of

the workmanship, depends the excellence of the work it is able to perform.

The two principal models, upon which nearly all stands are made, have been named the "Ross model" and the "Jackson model," both of which may be studied in the productions of Messrs. Ross & Co. Fig. 5 shows the Ross model, in which the body is attached by its base to a transverse arm, which latter is borne on the summit of a racked stem, to be seen in the figure.

This is a very efficient and convenient form when the workmanship is good, and leaves nothing to be desired if the stem and transverse arm are sufficiently solid. At the same time, it is the worst model on which to make cheap instruments, as those who have used them will only be too ready to testify. A cheap stand made on the Ross model practically requires no fine adjustment, there being sufficient spring in the stem and arm to focus with, by slightly pressing upon the latter at its junction with the body.

In the Jackson model, shown in Fig. 6, the body has the rackwork attached to it, and is supported for a great part of its length on a solid limb, as shown in the figure. It is, therefore, much less liable to vibration than in the Ross model.

In a paper read before the Royal Microscopical Society in March, 1870, Dr. Carpenter detailed his experience of these two forms of instruments. He sums up as follows:— "My own very decided conviction is that the adoption of the principles of the Jackson model would be decidedly advantageous alike for first-class instruments, in which the steadiness of the image when the highest powers are being employed ought to be of primary consideration; for those second-class instruments which are intended, at a less cost, to do as much of the work of the first class as they can be made to perform, portability here being of essential importance; and for those

third-class instruments in which everything has to be reduced to its simplest form, so as to permit the greatest reduction in their cost."

Fig. 6.

This summary of Dr. Carpenter's is so complete in its apology for separating microscope stands into three classes, that we will not add one word more.

The two different patterns made by Messrs. Ross, and shown in Figs. 5 and 6, are furnished with an extremely solid foot, which is cast in one piece. The "coarse adjustment" may be easily understood from the illustration, and the "fine adjustment" is obtained by the action of a milled head upon a lever which moves the nose-piece, in either case. Both instruments are furnished with a centering and traversing substage, and also with a rotating movement which is worked by a rack and pinion. The stage itself is very complete, the object slide when laid upon it can be instantly secured in position, and the whole stage with the object *in situ* can be rotated round the optical axis as a centre. The circular motion is graduated, and thus answers many useful purposes. A rectangular motion in two directions can be imparted to the stage by means of two milled heads.

In the most recent forms of instrument the stage is made very thin (so far as is consistent with steadiness), and the central opening large, so as to admit light of great obliquity, for which purpose the mirror is placed at the end of a jointed arm, so that it may be considerably extended.

The large best microscope of Messrs. Powell and Lealand is very heavy and massive; Dr. Lionel Beale speaks of it in high terms of praise. The focussing movement, is however, upon the Ross model, but the pattern itself is quite unique, and though of different construction to other forms, is said to respond satisfactorily to the requirements of the microscopist.

The stage has one inch of motion in two directions, by means of the milled heads, and is capable of rotation round the optical centre by means of a wheel and pinion. This is combined with a very thin stage for oblique illumination of

objects, either by means of the mirror or the achromatic prism. There is also a graduated silver circle, which can be used as a goniometer or for a variety of purposes. The substage has rotary, vertical, and rectangular motions, and both stages have for their foundation a solid brass ring, firmly attached to the stem.

Messrs. R. & J. Beck make many forms of microscopes, but their "Pathological," shown in Fig. 7, is perhaps the most useful to the real worker. It has been specially designed with a view of applying everything necessary for clinical and bacteriological work to a small instrument. It has a lever fine adjustment, and a rack and pinion substage with centering screws, carrying a wide-angle achromatic condenser, accurately corrected for spherical aberration. The stage is of glass, and therefore not mechanical, but for the kind of work it is intended for this is no drawback.

This chapter is not intended to be merely a catalogue of microscopes, but is designed to show the would-be purchaser the various forms there are in the market to aid him in the task of selection. It is not our intention to elevate or disparage the productions of any maker in particular, but we do advise the beginner to be very careful in the selection of an instrument, as we have nearly always found, that if anyone has rushed blindly into the purchase of a microscope, on the recommendation of a friend, sooner or later he has been dissatisfied with it and has finally purchased another of his own choice.

It is a good plan before deciding to purchase, to attend several of the microscopical soirées now so popular during the winter months. The would-be microscopist can there see many patterns of instruments and will be able to gain some valuable information by talking over their merits and demerits with the possessors. The exceedingly large stands will not be found very handy for every-day work, they are

Fig. 7.

FIG. 8.

useful, nay, almost imperative when working with high powers, or for micrometrical research, but the medium size stands will always commend themselves to the average worker.

The "Challenge" stand of Messrs. Swift & Sons is shown in Fig. 8, and has found much favour during the past few years having gained the reputation of being a good working instrument. The rackwork of the coarse adjustment has lately been much improved, and new devices adopted for working the fine adjustment which have added to the value of the instrument. Messrs. Swift have recently adapted several features of the "Wales" model in one of their newest stands, and they intend making this their speciality in future. This new pattern will be figured when treating of microscopes of special pattern.

Mr. Henry Crouch is well known in England as a producer of several stands of excellence. All his microscopes are now made with spiral rack and pinion to the coarse adjustment, an improvement which Mr. Swift was the first to introduce; the mirror stems are pivoted from the attachment to the limb so that they can be brought up level, with, or above the stage, and in the "Premier" and "Students" stands the stages have been enlarged and their thickness reduced, and the substage fitting has been made to swing aside, leaving the stage entirely clear for oblique illumination by means of the mirror. Fig. 9 shows the Crouch No. 1, "Jackson Model" stand.

Fig. 10 will serve to illustrate a class of microscope largely used by students of Histology, who wish for a very cheap yet reliable instrument. The coarse adjustment is effected by sliding the body in a cloth-lined tube, but if required a rack and pinion adjustment can be added. Most of the makers of microscopes furnish a similar quality of stand suitable for class purposes, and we are glad to say

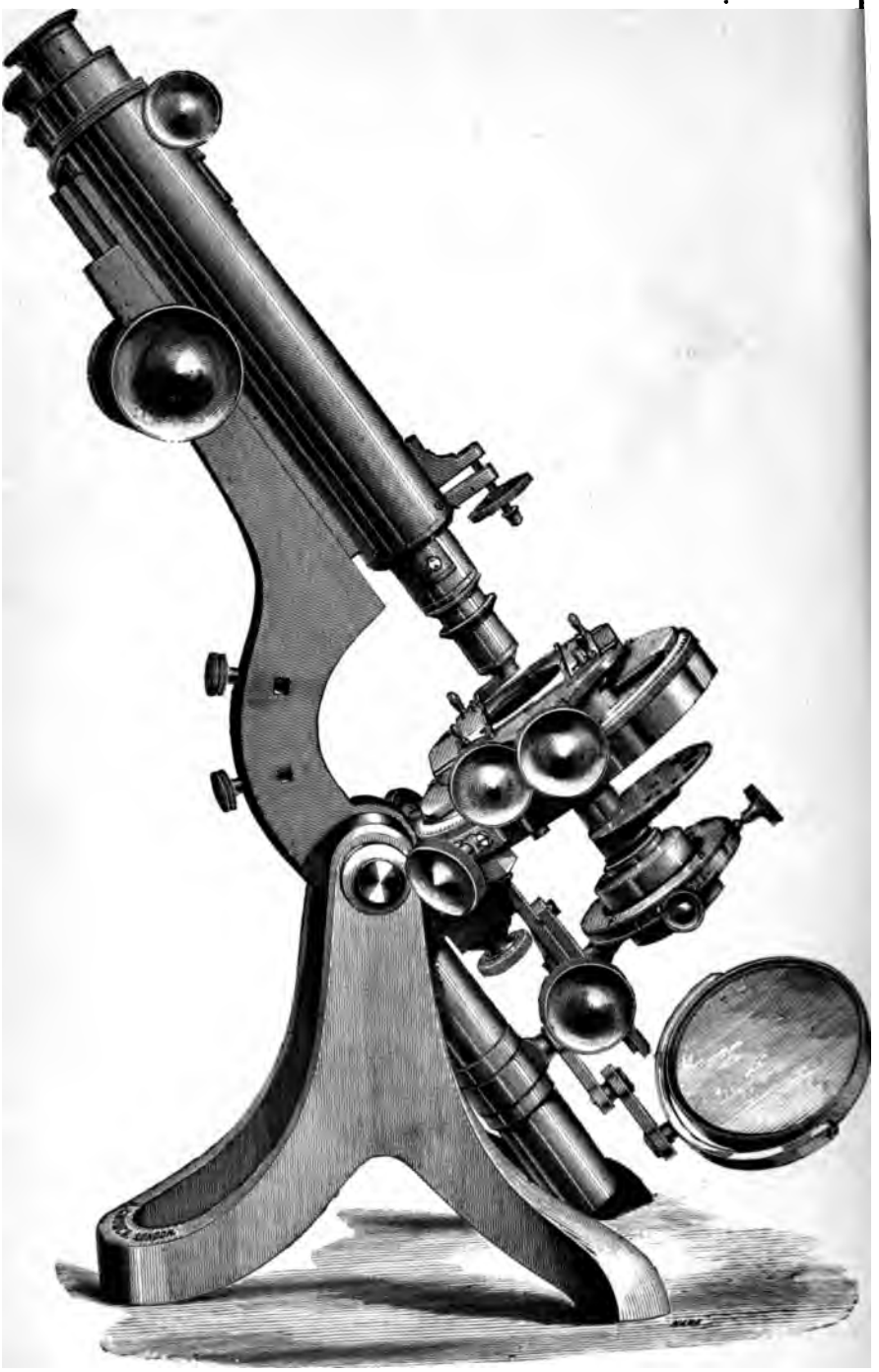


Fig. 9.

that no one in this country produces the cheap vertical microscopes, with non-inclinable bodies, the use of which tends so much to damage the eyesight.

Fig. 10.

Fig. 11.

Collins' "Harley" binocular, shown in Fig. 11, is now constructed on the Jackson model. The base is large and made in one piece, the limb carrying the body, stage, substage, and mirror is in one piece also, with a machine-

FIG. 12.

planed groove from end to end. A rackwork of six inches in length is attached to the body so that very low power objectives can be used. The fine adjustment is placed between the tube and the limb. The stage is circular in

form and with concentric rotation, with a simple arrangement for adjustment should it get out of the centre. It has a clear aperture underneath, of three inches, when the apparatus plate is removed, and consequently very oblique illumination can be obtained by means of the mirror alone. Collins' "Histological" is a very handy little instrument.

Mr. John Browning formerly constructed all his stands upon the Ross model, but he has recently issued a new pattern, built on the Jackson model, an illustration of which is shown in Fig. 12. Having seen and worked with this stand the author can testify to its steadiness, and it is not too heavy to carry about to Society meetings.

We cannot pass this description of microscopes without calling attention to the productions of Carl Zeiss, of Jena. Fig. 13 is an illustration of his largest and best stand, which is a paragon of excellence so far as workmanship is concerned. To our eye the pattern is too harsh and severe, but for actual work where a large stand is necessary this one can scarcely be excelled. Its excellence mainly lies in the method of mounting the substage, condenser, and other fittings, whereby they can be turned aside at a moment's notice. The body tube is short but the draw tube allows of the use of the objective at the standard English length. It is a great convenience, especially in photo-micrography, to be able to shorten the body tube.

The Zeiss stand, No. IV., which is shown in Fig. 14, is a much more portable instrument than the foregoing, and is the one having the greatest sale. It is shown in the drawing with the condenser removed, and will be found a very good instrument for actual work, the more especially for photography with the microscope.

Mr. Charles Coppock, formerly associated with the firm of Messrs. Beck, has issued a microscope suitable for science teaching, and constructed mainly from data obtained from

Fig. 13.

Fig. 14.

consultation with the leading teachers of science in Edinburgh. The stage and body are carried on a turned pillar, as shown in Fig. 15, after the style of the

Fig. 15.

continental models. The body tube is short, but the draw tube enables the standard length to be used, a stop indicating when it is drawn out to this length. The coarse adjustment

is obtained by a sliding motion in a tightly fitting tube, while the fine adjustment is manipulated with a milled head screw in the ordinary way.

Mr. Coppock is also the English agent for Nachet, of Paris, whose instruments, of the kind generally used here, are shown in Figs. 16 and 17. M. Nachet has obtained a good

FIG. 16.

FIG. 17.

reputation for his optical work in connection with the microscope, of which we shall have again to refer when treating of binoculars.

After all, a microscope stand must satisfy certain conditions, and if these be fulfilled it scarcely matters to the

owner who the maker has been. It must be made of good hard brass, be furnished with a heavy foot, and well balanced, so that it may be placed in any position without overturning. All the rackwork and screws must move easily, firmly but not stiffly, and without "loss of time" or "backlash." Stops should be placed so that the body may be set either horizontally or vertically as required, and plane and concave mirrors should be always provided, preferably on a jointed arm. The lower extremity of the body should be furnished with the Society's screw, and *no microscope should be purchased which has not this thread.*

A mechanical stage is not absolutely necessary but is a great convenience, despite all opinions to the contrary, and some accessories cannot be used without it. A circular and rectangular motion should be capable of being imparted to the stage without the intervention of rackwork; but a duplicate motion by means of rack and pinion should be supplied to the better instruments. Reasons for the rotation of the stage will be found in the chapter treating on the use of the polariscope.

The medium and best stands should be furnished with an adjustable substage fitting capable of being moved vertically by means of rack and pinion; but the cheaper kinds are usually provided with a removable tube, into which the polariscope, condenser, and other accessories are made to fit. These should be capable of taking accessories made to the one and a half inch gauge, even if purchased from different makers.

It is much to be regretted that a specified size of body tube is not recognised by microscope makers. At present the oculars of one maker will not always fit the tubes of another, though it is satisfactory to remember that the largest tube will always take a smaller ocular, and that a small tube will not take anything larger. According to the measurements of the

author, the following are the inside diameters of the tubes of several makers, carefully measured with vernier calipers.

Name of Vendor or Maker.	Description of Model.	Diameter of Ocular.	
		Body.	Neck.
		Inches & 64ths	Inches & 64ths
Powell & Lealand...	Large Stand.....	1·23½-64	58-64
R. & J. Beck.....	National	1·17-64	57-64
Smith & Beck	Large Stand..... ...	1·17-64	56-64
Ross & Co.....	Ross-Jackson	1·20-64	57-64
,, ...	Ross-Zentmayer	1·20-64	57-64
Dancer	Large Stand.....	1·17-64	57-64
Browning	Ordinary Stand.....	1·17-64	54-64
Swift	Challenge.....	1·13-64	56-64
Collins	Harley	1·17-64	
Crouch	Premier	1·14-64	54-64
Aylward.....	Working	1·23-64	56-64
Parkes	Students.....	1· 7-64	*
Engelbert.....	Ordinary	1· 6-64	*
Zeiss	,, ...	58-64	*
Leitz	,, ...	59-64	*

Upon those stands marked with an asterisk the camera-lucida is not made to fit in the ordinary way.

Another cheap instrument, originally registered by Mr. Swift, which is now furnished by several makers, is shown in Fig. 18; it has a sliding body for coarse adjustment, a fine adjustment, draw-tube, wheel of diaphragms, tube fitting for substage apparatus, plane and concave mirrors, one eye-piece, 1-inch and ¼-inch objectives, and stand condenser, in mahogany case, the price of which is 5*l.* 12*s.* 6*d.*, to which can be added afterwards a polariscope, 25*s.*, a camera lucida, 6*s.*, and a stage micrometer, 5*s.* 6*d.*

Equipped with one of these instruments, which are remarkably steady, being hung on the Jackson model, the student is ready for an immense amount of work, and it is certainly much better to invest in an instrument such as this than to waste money on a cheaper instrument which has not the

Society's screw. In the purchase of a low-price stand, we would give the student the following advice:—Never purchase a cheap instrument made upon the Ross model; never purchase one which will not take objectives with the Society's thread without an adapter; never purchase an instrument without a fine adjustment, without it be for petrological studies merely; and above all, do not purchase the extremely trashy foreign separating objectives. If you want foreign glasses, there are good ones to be bought—buy them.

FIG. 18.

After all, there are a few objections to the form of instrument shown at Fig. 18. The body tube is narrow and the field lens of the eye-piece small in proportion, so that the field is limited in size; then, again, the absence of a coarse adjustment may for some purposes be found inconvenient, while the fitting below the stage should be made removable. On the other hand, the short tube gives a larger field when used for photo-micrography, and is also more convenient as a dissecting microscope, lengthening as it does the anterior conjugate focus and giving more room for the needles.

Recognising these and several other advantages, the author had one specially constructed, as shown in Fig. 19. The draw tube is wide enough to take the full-sized eye-pieces,

and when it is fully extended the whole forms a body of the ordinary length. There are coarse and fine adjustments, a diaphragm in the thickness of the stage, plane and concave mirrors, the whole costing the author the moderate sum of three guineas and a half.

With this form one is not bound to bad or medium eye-pieces and objectives; they can be bought separately and selected. Several enterprising manufacturers make the instrument as shown in Fig. 19, and sell it for five guineas with an A eye-piece and a good inch objective. The details of this instrument are as follow: — When standing vertically and closed down, the top of the eye-piece is 11 inches from the table, the collar in which the body slides being 3 inches in length, and lined with velvet. The body is 5 inches in length, and the draw-tube $4\frac{1}{2}$ inches, with an outside diameter of 1·3 inches, and is provided with an adapter for Continental eye-pieces; the stage is 3·7 inches from the table, and has a diameter of 3 inches; the mirrors are 2·2 inches in diameter, and the fine adjustment raises or depresses the entire body $\frac{1}{100}$ of an inch for each complete

Fig. 19.

revolution. The stage is less than ¼ of an inch in thickness. The author has found this a very convenient instrument for photo-micrography and for general microscopical work. The substage fitting will take all accessories made to the usual 1½-inch gauge.

Microscopes with Swinging Substages.

The instruments illustrated in the foregoing pages may be regarded as specimens of those in actual use by the majority of microscopists up to within the last few years. Of late there has been a departure from the ordinary construction, mainly with the view of improving the illumination of objects and of recording results so that an observation once made may be repeated without trouble.

In 1853 Mr. Thomas Grubb, Engineer to the Bank of Ireland, described, in the "Proceedings" of the Royal Irish Academy, how he had been able to observe, and restore any position of illumination, by mounting a suitable illumination on a graduated, vertical, circular sector concentric with the focus of the microscope; and in a paper read in March 1858, before the Royal Dublin Society, he further described these improvements.

Nothing further seems to have been done in this direction until 1855, when M. Nachet, of Paris, constructed a traversing substage for M. Thury. This apparatus was specially designed to keep the focus of the illumination upon the object with varying degrees of oblique incidence. The movement was, however, from back to front, and not lateral; but in this device the mirror moved automatically and the

light constantly deflected into the axis of the condenser whatever may have been the inclination.

In 1862 Dr. Royston Pigott adapted a semi-circular arc, carrying a condenser, to a double motion stage, placed beneath the upper stage movements, and by this means particular angles of illumination could be readily attained and recorded.

Following in the wake of these experts, Tolles, of Boston, U.S.A., in 1871, constructed a small microscope stand, fitted with a radial arm beneath the stage, carrying a condensing lens of about 1 inch focal length, so arranged that by deflecting the arm any degree of obliquity of the illuminating beam could be obtained. In 1875 he seems also to have adapted a circular track, having its centre coincident with the object on the stage, to a microscope stand belonging to Dr. Bacon, in order to obtain the before-mentioned advantages without reconstruction of the instrument.

In 1873, Mr. Bulloch, of Chicago, designed his "Sector" microscope, which was exhibited in that year. In this instrument the sectorial arc was placed below the stage, and the mirror was fixed to a separate arm, which could be swung over the stage if desired.

From this time much energy was displayed in the United States in producing stands having advantages over the older forms. In April, 1876, Zentmayer, of Philadelphia, first exhibited his "Centennial" Microscope, shown in Fig. 20.

This stand is full 19 inches high when arranged for use. It is mounted on a broad tripod base with revolving platform, bevelled, silvered and graduated in single degrees for measuring the aperture of objectives. Upon this platform two pillars are planted, between which the bar and trunnions (which are of one piece), swing for inclining the instrument to any angle.

The coarse adjustment is effected by rack and pinion.

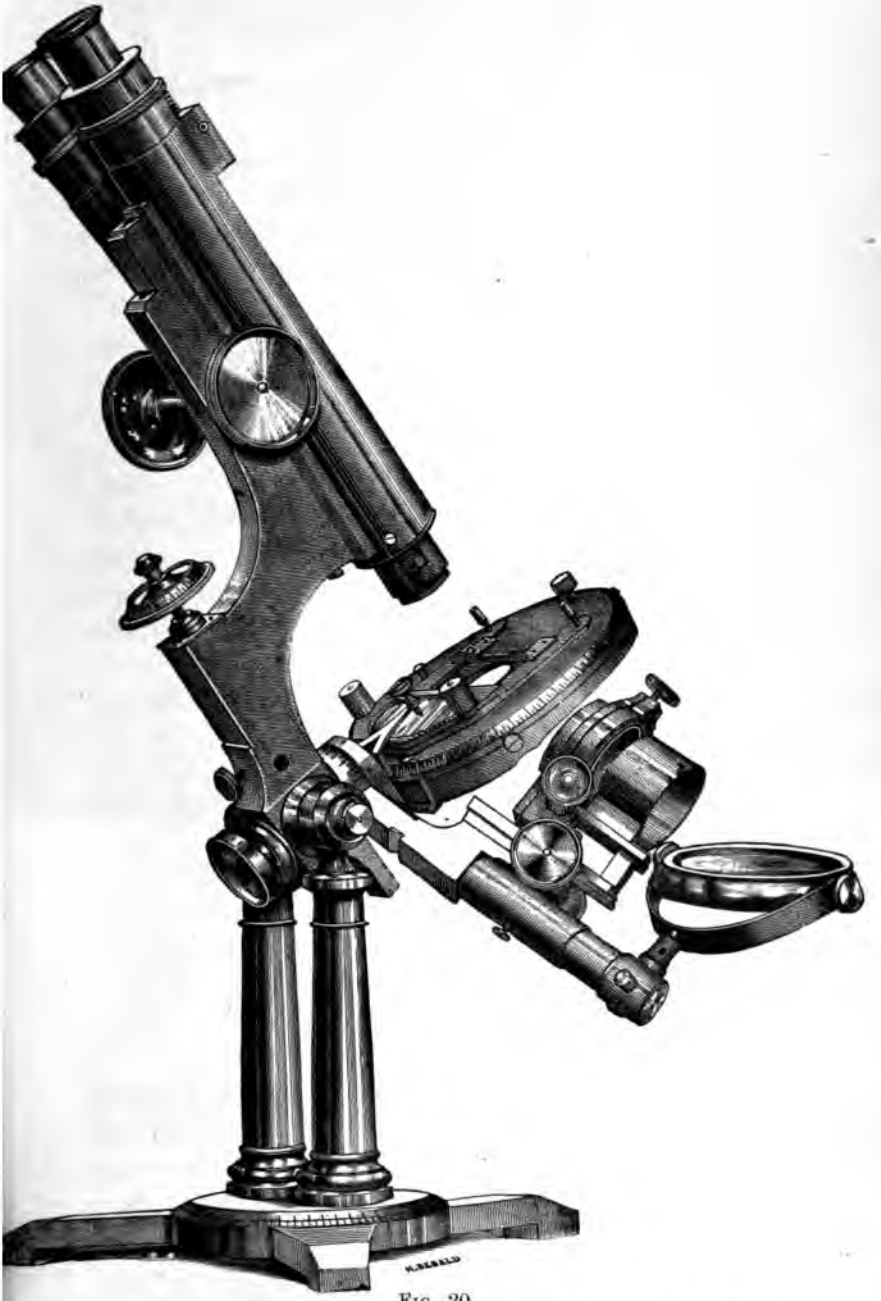


FIG. 20.

The fine adjustment is removed to the more stable part of the instrument, the bar, which is provided with two slides, one for the rack-and-pinion adjustment, and close to it, another one of nearly the same length, for the fine adjustment, moved by a lever concealed in the bent arm of the bar, and acted upon by a micrometer screw. In this way the body is not touched directly when using the fine adjustment, and the body does not change the relative distance of objective, binocular prism and eye-piece.

The swinging substage, which carries the achromatic condenser or other illuminating apparatus, and the mirror, swings around a pivot placed behind the stage, of which the axis passes through the object observed, so that the object is in every position in the focus of the illumination. Although provided with but a *single* joint, it admits of being swung *over any of the stages*, a complete revolution is only interfered with by the body of the instrument. It is provided with a graduated circle at the upper collar for registering the degree of obliquity, and a stop to indicate when it is central with the main body.

As an object placed on the stage is in a plane with the axis of the trunnions, it is obvious that, if the instrument is placed in a horizontal position, the object is in the axis of revolution of the graduated platform, and the angular aperture of an objective focused on this object can be easily measured. It can be readily seen that in this position the object is in the centre of all the revolving parts of the instrument, the revolving stage, swinging substage and the platform.

The principal stage was devised in 1862. It consists of a bell-metal ring, firmly attached to the bar, but adjustable by means of set screws, in order to make it perfectly concentric to the optical axis of the instrument. This ring receives the stage platform, which has a complete revolution. The outer edge is bevelled, silvered and graduated to serve as a

goniometer. The carriage on which the object is placed rests on a piece of plate-glass, kept down by a spring with an ivory-pointed screw to the two rails on the revolving stage platform, which gives an exceedingly smooth and firm movement, and a freedom of motion not obtained by any other arrangement. The stage may be detached with facility, by simply unscrewing the nut at the back of the bar, to be replaced by another stage as, for instance, a mechanical stage.

For those who prefer mechanical stages, an adjustable, graduated and revolving mechanical stage is provided. It is 5 inches outside diameter, the lateral movement, which is accomplished by a screw, has a motion of $1\frac{1}{4}$ inch, and the longitudinal slide, by rack and pinion, allows $1\frac{1}{8}$ inch. Although very steady it is extremely thin, admitting an obliquity of full 70°. Both stages are reversible on the stand, thus admitting of unlimited obliquity and still keeping the object in the centre of the swinging bar.

The substage is divided into two cylindrical receivers, to facilitate the adaptation of several accessories at one and the same time. The upper cylinder has *centering adjustment*, the lower cylinder of the two can be moved up and down, or entirely removed.

Fig. 21 shows Zentmayer's Histological stand, which is an adaptation of the swinging substage to a cheap form of microscope.

The entire instrument is made of brass. The base and uprights are one piece, of a peculiar shape, of great rigidity, to which the bell-metal bar is attached by a joint, allowing the use of the instrument in any angle of inclination; perpendicular and horizontal positions are indicated by stops. The coarse adjustment is accomplished by a sliding tube, or by rack and pinion; the tube is $5\frac{1}{2}$ inches long, capable of elongation to the standard length.

The fine adjustment is of the same style as the one of the American Centennial Stand. A concealed lever is acted upon by a micrometer screw and moves the entire body,

FIG. 21.

which is fitted to the grooved bar, giving a steady and delicate movement. The arrangement of the swinging substage and mirrors is the same as in the United States Army Hospital Stand.

The removable substage carries the diaphragms, which can be shifted up close to the object.

The stage is a modification of the glass stage, and consists of a glass bar kept down by two spring clips against which the object rests. By this method the object may be moved in the latitude, and the longitudinal movement is accomplished by hand. The spring clips may be used independently for holding anything in a fixed position, by simply placing them in the extra holes provided for that purpose. The stage is only three inches from the table when in a horizontal position.

Messrs. Ross & Co., of New Bond Street, London, whose name has been mentioned in connection with the older forms of the microscope, have remodelled their instruments, and have adopted all the leading features of the Zentmayer model which, together with a few improvements of their own, constitute the "Ross-Zentmayer" microscope. The largest form of stand is shown in Fig. 22; it needs no description, as all that has been said regarding the Zentmayer "Centennial" applies to this equally, with the exception that the pillars do not revolve upon their base.

In 1877 Bulloch, of Chicago, brought out his "Congress" stand, which was patented two years later. A section of this instrument is shown on page 1074 of the *Journal of the Royal Microscopical Society* for 1880; subsequently, however, the instrument was improved and several additions made to it. Fig. 23 shows the latest form of this microscope, and it will be seen that it differs from the Zentmayer, Ross-Zentmayer, and some other models, in that the condenser or mirror may be individually rotated round the object as a centre, and, furthermore, there is no connection between the substage or mirror, and the stage itself, which is fixed to the limb by an angle piece quite independent of the swinging arms.

The end of the tube is fitted with the "Butterfield" broad

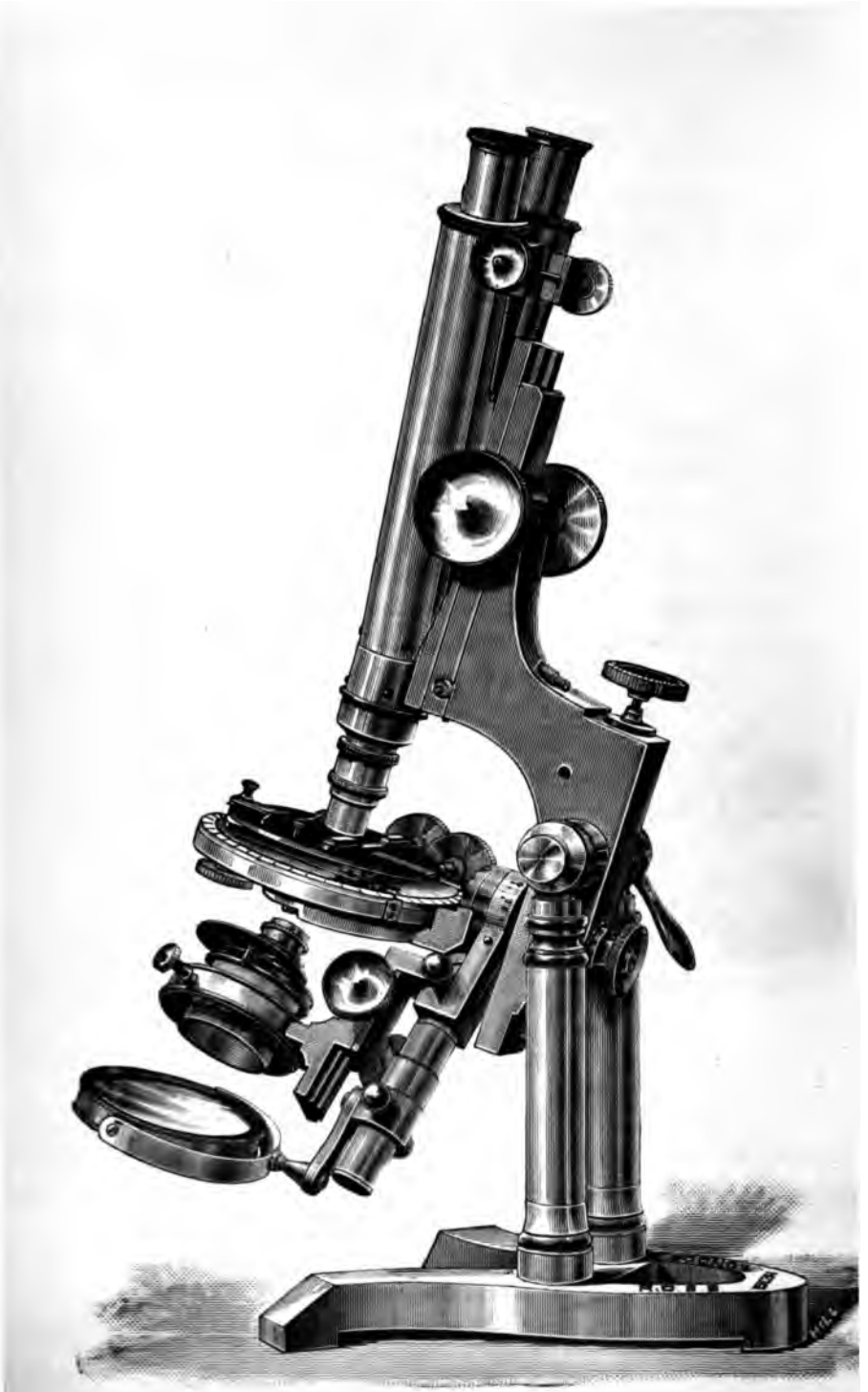


Fig. 22.

FIG. 23.

gauge screw (one and a quarter inches in diameter) for low power objectives of high angle, and in this screw are two separate nose-pieces carrying the "Society" thread, one is for the binocular, which must have diaphragms, the other has a clear aperture, the diameter of the Society's screw.

The "Biological" stand, made by Mr. Bulloch, is a small instrument of which we can only speak in high terms of praise; it was patented in 1879, and is the outcome of much experience with the "Congress" stand. This microscope is 12½ inches high, and the stage is 3½ inches from the table; the body is 5 inches long, and the draw tube also 5 inches in length, marked with a ring to show the standard length. The diameter of the body tube measures 1·4 inch. The stage has a revolving concentric movement, the mirror and substage can be made to swing over the tube, and there are spring stops to indicate when they are in a line with the axis of the instrument. The lower end of the main tube is fitted with the Butterfield broad-gauge screw, forming an adapter carrying the Society thread. The price of this instrument in walnut case is, with one eye-piece, $40, equal to about £8 8*s*. of our money, or with ¾-inch and ⅕-inch objectives, £12. It is shown in Fig. 24.

In the year 1877, Tolles designed a microscope for Dr. Blackham, in which a large graduated disc was attached to the main limb of the instrument. The substage was fitted to a zonal carrier near the edge of the disc, which could be moved circularly by means of a milled head. The stage was fixed to the centre of the circular disc. This microscope was figured in Charles Stodder's catalogue (Tolles' agent), and described by him there.

Messrs. Sidle and Poalk, in 1880, introduced their "Acme" microscope with swinging substage, which was one of the earlier forms of the cheaper instruments with this adjunct, and since that date the majority of American instru-

ments have been constructed, so that the radial arm carrying the mirror, or substage, or both, may be revolved in a circle, or nearly so, with the object as its centre.

Fig. 24.

Messrs. R. and J. Beek have also incorporated Grubb's principle in the construction of their best stand, which they call the "International," and shown in Fig. 25.

This instrument has a heavy brass tripod for its base, upon which is placed a revolving graduated fitting, from which rise two pillars, together forming the foot. Between these pillars is hung the limb which carries the body at its upper end. In its centre the compound stage is fixed, beneath which is a circular plate carrying the swinging bar and substage.

The stage itself is attached to the limb on a pivot, and can be set to any angle of inclination, the plate being divided, so that the angle may be recorded, and in this way also, light of any degree of obliquity may be used without interference from the thickness of the stage. Horizontal movements by two milled heads are capable of being imparted to the stage, which may also be revolved by means of a milled head, or when this is pulled out, by using the two ivory studs fixed to the top plate for that purpose.

The swinging substage is mounted perfectly true with the body, and is moved up and down in its fitting by means of milled heads. In this fitting all the varied appliances for modifying the character and direction of the light are fitted. The bar into which the substage fits is itself attached to an arc working in a circular fitting, and is capable of rotation by means of a milled head, so that it may be carried round and above the stage if necessary. The amount of angular movement may be recorded from the graduated circle. The lower triangular bar carries the mirror when the illumination is required to be concentric with the optical axis of the instrument; when desired, it can be made to slide on the substage bar, and then can be moved above or below the stage in the same manner as the substage.

The main difference between the Tolles-Blackham stand

Fig. 25.

and that of Messrs. Beck is that the vertical disc in the latter is not fixed to the main limb as in Tolles' pattern, but is provided with a vertical sliding motion to allow for different thicknesses of the object slide, so that the motion of the swinging substage may be strictly concentric with the object undergoing examination.

In the States, all the principal microscope makers have embodied the principle of the swinging substage in the construction of their instruments. Messrs. J. W. Queen & Co., of Philadelphia, supply a series of "Acme" microscopes of which we give an illustration of No. 3 in the series, as Fig. 26, which is a favourite instrument there.

The entire microscope is of brass, highly finished. The draw-tube is turned out larger inside below the top, so that the long eye-piece does not rub off the black finish, hence there is no bright surface to cause reflection with the shorter eye-pieces. The eye-pieces are designated by their equivalent focal length, as are objectives; thus the (approximate) powers given by various combinations are easily calculated. The body-tube has the "broad-gauge" screw for low powers of excessive aperture, in addition to the standard society screw.

The rack-and-pinion adjustment has a long range. The fine adjustment is a very delicate and truly working one, suitable for the highest powers. The body is firmly carried, upon rollers, and is moved by a lever actuated by a screw at the rear of the arm. The head of screw is graduated so that measurements of thickness may be made directly or by the application of a simple formula.

The stage is of black glass, with movable object-carrier held down by two ivory points, and has complete rotation. A plain brass stage with rotating stage-plate and spring clips (as in the former model) is furnished instead of glass stage at a less price. Both stages have a standard screw-thread in the lower plate for the reception of selenite, which can be

rotated independently of the Nicols prism. Other accessories also may be thus fitted if desired (the hemispherical lens for example).

FIG. 26.

The substage is movable on the mirror bar, is of the gauge most usually adopted, and carries an adapter having society screw, for the use of objectives as achromatic condensers. To this is screwed an iris diaphragm, conveniently shaped like a short objective. This may be placed close to the object or at any required distance.

The mirrors (plane and concave) are also adjustable on the mirror bar; the usual position is at the focal distance of the concave mirror from the object. Mirrors alone or with the substage may be swung obliquely or above the stage. A graduated disk at the back of the stage registers the degree of obliquity, and may be used for the measurement of the aperture of objectives.

Both stage and substage are susceptible of centering adjustment by loosening the capstan-head screws which hold them in position, centering by hand, and tightening again by a steel key furnished with the instrument.

The base (which is somewhat larger in the binocular form) is firmly attached to the pillar by a thumb-nut. The advantage of this arrangement is, that it may be rotated in order to support the weight to best advantage in different positions of the body; thus, when the latter is vertical the toe should be placed forward, and this position is generally the best.

Wear of moving parts is provided for throughout, and the adjustments may in many cases be made by the owner.

There is an extra nose-piece furnished with the binocular, so that it (as a monocular) may be used with objectives of the very widest aperture consistent with the society screw, without any reduction of aperture by diaphragms.

The Bausch and Lomb Optical Company, of Rochester, and New York City, are also makers of some very excellent microscopes, of which might be mentioned the "Universal" stand shown in Fig. 27.

The base is of the tripod form and made of brass; it has on its lower surface three soft rubber pads, and is sufficiently heavy to sustain the instrument firmly at any inclination of the

FIG. 27.

body. The brass pillar is large and heavy, and connected by joint for inclination of the arm. The coarse adjustment is by rack and pinion and of sufficient range to admit of the use

of the lowest power objectives; the fine adjustment is by micrometer screw acting on a patent frictionless motion. The main tube has two draw-tubes, by which a considerable range in length may be attained; they may be contracted to less than the standard, to decrease the height of the instrument when used in an upright position, and may be extended beyond it to increase the magnifying power; both draw-tubes have society screw, and the main tube has broad gauge screw and adapter for society screw. The stage has concentric revolving motion with removable spring clips, and its upper surface lies in the same plane as centre of mirror-bar movement and joint for inclination; it is thin to allow the greatest obliquity, but firm under any manipulation. The mirror and substage bars move independent of one another or together, and while the mirror-bar swings to allow the use of the mirror at any possible angle below or above the stage, the substage bar revolves completely around it and may be placed between the stage and the arm, where various illuminating accessories may be used; in this position the substage may also be entirely removed, which leaves the mirror alone in its relative position to the stage; the mirrors are of large size, and both these and the substage are adjustable on their respective bars; the circular bearings of these are large and are graduated to degrees and silvered. A steel pin for centering the stage and substage accompanies the instrument.

The price of this stand with one ocular, in polished case, is $55, or with extra ocular, ocular micrometer, camera lucida, $\frac{3}{4}$-inch and $\frac{1}{5}$-inch objectives, $80.

Another stand, made by the same firm, and which may be compared with the working microscope, shown in Fig. 19, is the "Harvard" model, illustrated by Fig. 28, and in our opinion it is sure to command a large sale.

The stand is made entirely of brass and so proportioned

that it will give the most effectiveness to the various parts. Although constructed so as to allow its convenient use in an upright position on an ordinary table, it still gives sufficient range of adjustment for all ordinary work. The base is of the horse-shoe form with a projection at the back to give it

Fig. 28.

steadiness when the body is inclined. Coarse adjustment is by a sliding tube in a sleeve, provided with a spring which reduces the friction and retains a smooth movement; fine adjustment is by micrometer screw with milled head of more

than ordinary size, acting on a patent movement. The main tube has a draw-tube with a mark indicating standard length. The stage is large and stiff and provided with spring clips; an Iris diaphragm is attached to its lower surface in such a manner as to allow its centering and entire removal for oblique light. The mirrors are plane and concave and swing with the mirror-bar to any obliquity below the stage and above it for the illumination of opaque objects.

Although we have only mentioned the Bausch and Lomb Optical Company, in connection with the smaller stands, we have no desire to infer that the larger and more complete instruments cannot be obtained from them. In fact, the contrary is the case, they have some very interesting specialities, and for large and complete stands their "Professional" microscope leaves little to be desired for the actual worker, while their pattern known as the "Concentric" will be noticed farther on.

Microscopes of Special Pattern.

A few years ago the firm of Messrs. Watson & Sons, of Holborn, patented the arrangement, an illustration of which may be found in Fig. 29. In this form, the mirror is accurately centered, while the body of the instrument, together with the stage, is made to incline in order to arrive at the same results as attained with the foregoing accessories.

It will be seen that the new stand enables the observer to examine an object as he would if it were held in the hand, and viewed by the naked eye—that is, to turn it about in every possible way towards a ray of light proceeding in a fixed direction—and so, without once losing sight either of the light or the object, to observe its appearance when illuminated by light of every degree of obliquity.

This is the fundamental idea underlying its construction, and in this consists the great difference between it and the old forms of stand (although it has all the uses of the latter), where the object remaining fixed, the only way in which its illumination can be varied is by moving the illuminating ray—which, in the amount of the results it affords, and the amount of time it consumes, is stated by the inventors to be in every way inferior to the new one.

An inspection of the engraving (Fig. 29) will show how this idea is worked out. On the top of a strong pillar, to which it is attached by a massive cradle-joint allowing of inclination in a vertical plane, is fixed the arm carrying the body, which latter is provided with rack adjustment, and a new and improved fine adjustment, rendering unnecessary the usual often unsatisfactory loose nose-piece. The stage is so fixed with regard to the arm that the object when lying upon it is in a line with the centre of the cradle-joint, so that upon inclining the body the object moves with it, and is presented at every possible verticle angle to a ray proceeding to it from a given direction. The stage is of a new and improved construction, being exceedingly thin—in fact, the thinnest mechanical stage yet devised—and is capable of giving a complete rotation of the object.

Beneath the stage swings the substage arm, concentric with the object, and carrying the usual screw centering and rack adjusting substage.

Behind the substage arm is a strong bar, provided with a dovetailed groove, into which the mirror bar slides. This is so pivoted to the substage arm as to allow the latter to be swung aside and the mirror used alone when requisite, without the trouble of taking the substage away altogether. This is a great advantage, as it permits the substage, and any apparatus it may be carrying, to be swung into or out of position in a moment with the mirror in the position here

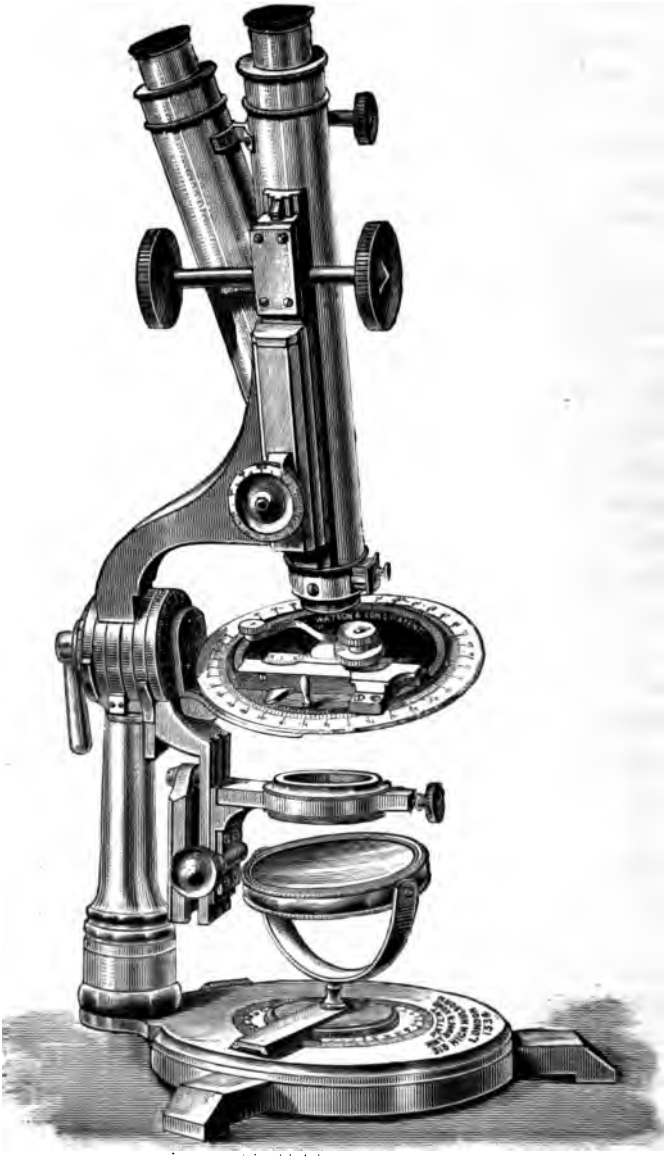


FIG. 29.

indicated. The stand has all the uses of the old forms of microscope, and can be employed in exactly the same way, but even then its peculiar motions round the object as a centre give it very great advantages in every class of investigation. But it is when the mirror occupies the position now to be described that the peculiar properties of the new stand are brought fully into play.

The upright pillar, carrying the body and stage, is attached at its foot to a massive circular plate, carrying a graduated circle which rotates round a point exactly beneath the centre of the stage; and moving independently and concentrically with this is another smaller circle, having a dovetailed groove ploughed across it, into which the mirror bar can be slid when withdrawn from the substage arm. A spring catch attached to the dovetailed circle falls into a notch in the mirror bar when the centre of the mirror is exactly beneath the centre of the stage. This is the most useful position for the mirror, as a ray falling from a source of light upon it may be reflected upwards perpendicularly upon the object, when the body of the microscope is vertical, then without interfering again either with the mirror or lamp, or interposing any accessory apparatus whatever, but simply by inclining the body, the light falls upon the object with a gradually increasing obliquity until, when the instrument is nearly horizontal, a perfect dark-ground illumination can be obtained even with the highest powers, while the gradual way in which the light becomes more and more oblique immediately under the eye, and the capability of arresting the inclination at that point where the most suitable illumination for the object under examination is obtained, give to the observer powers he has seldom before had at his command in any form of microscope yet produced.

The horizontal rotation mentioned above allows the object to be directed to the light at every angle in azimuth—to

borrow a term from the astronomer—as the cradle-joint on the top of the pillar gives every angle in altitude; the object occupying the centre of both motions, by the combination of the two it can of course be placed in every possible position. These angles are read, the latter by a graduated circle in the outer side of the cradle-joint, giving the inclination of the body to the vertical, the former by means of the graduated circle at the foot; readings of these circles being taken with the mirror placed as above described at any time by so fixing the instrument that these circles read the same. Any desired effect will be exactly reproduced, *wherever the lamp may be placed*—a point of the greatest importance to workers with high powers.

There is a third divided circle on the substage axis, giving the inclination of the substage to the axis of the body. A strong clamp on the outer side of the cradle-joint holds the body firmly at any inclination, and a graduation on the slide of the coarse adjustment enables the working distance of the objectives to be measured and compared.

Time alone can show whether these present apparent advantages are lasting, or whether the instrument will wear as well as those of the old form of construction.

Recognising the advantages which these new motions gave to the operator, Mr. F. H. Wenham (whose name is so well known for his improvements to the microscope) devised the special form shown in Fig. 30, for the purpose of obtaining the maximum range of illumination in all directions, and this is attained by causing all the movements of inclination and rotation to radiate from the object as a common centre. Seven radial motions are here combined:—

The inclination of the whole instrument (except the base) from the perpendicular to the horizontal, by means of a sector sliding between jaws attached to the upper base-plate. The lateral inclination of the limb to either side, carrying with it

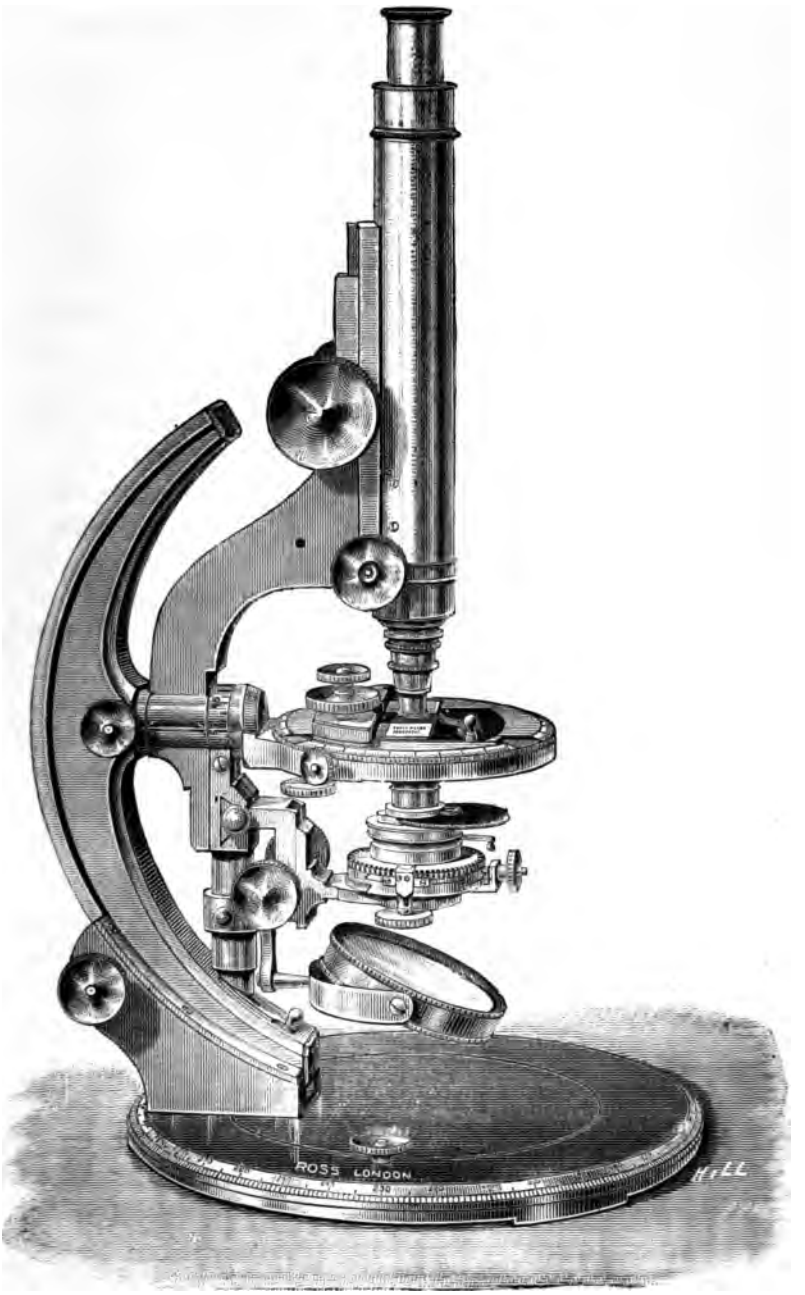


FIG. 30.

the tail-piece, or of the limb and stage only, the tail-piece being clamped to the sector. With the sector clamped at the usual angle, this latter arrangement furnishes very ready and practical means of varying the illumination in altitude from 0° to nearly 90°, either direct from the mirror or combined with a low-power condenser. The rotation of the whole instrument on the lower base-plate on an axis which is the prolongation of the optic axis when the instrument is vertical, as shown in the figure. The swinging of the tail-piece, suspended on an axis, the centre line of which passes through the object on the stage, cutting the optic axis at right angles. The complete rotation of the mechanical stage upon the optic axis, secured by the milled-heads for the rectangular motions being placed on a vertical axis on the stage, and acting entirely within the circumference. This stage can be easily removed, and may be replaced by a glass or other stage. The complete rotation of the mechanical substage upon the optic axis. The partial rotation of the lamp, in azimuth, upon an arm pivoted in the centre of the lower base-plate, not shown in the figure.

An entirely new construction of fine adjustment is applied to this Microscope, consisting of a V-slide acted upon by two "snail"-cams, between the edges of which revolves a steel roller, forming the axis of, and actuated by a large milled-head passing longitudinally through the slide of the coarse adjustment, and projecting slightly on either side, in a convenient position for work. The V-slide is fitted within the body-tube, carries at its lower end the nose-piece, and is pressed downwards by a spiral spring, against which it is moved by the revolution of the cams.

The American Concentric Microscope (Fig. 31), was first made at the suggestion of the Hon. J. D. Cox, by the Bausch and Lomb Optical Company, and is constructed on the principle of the sliding, concentric motion of the body. Its main

advantage lies in the fact that its centre of gravity remains at about the same point over and close to the base with any inclination of the body and thus insures extreme stability. Its principle has been considerably commented upon, and

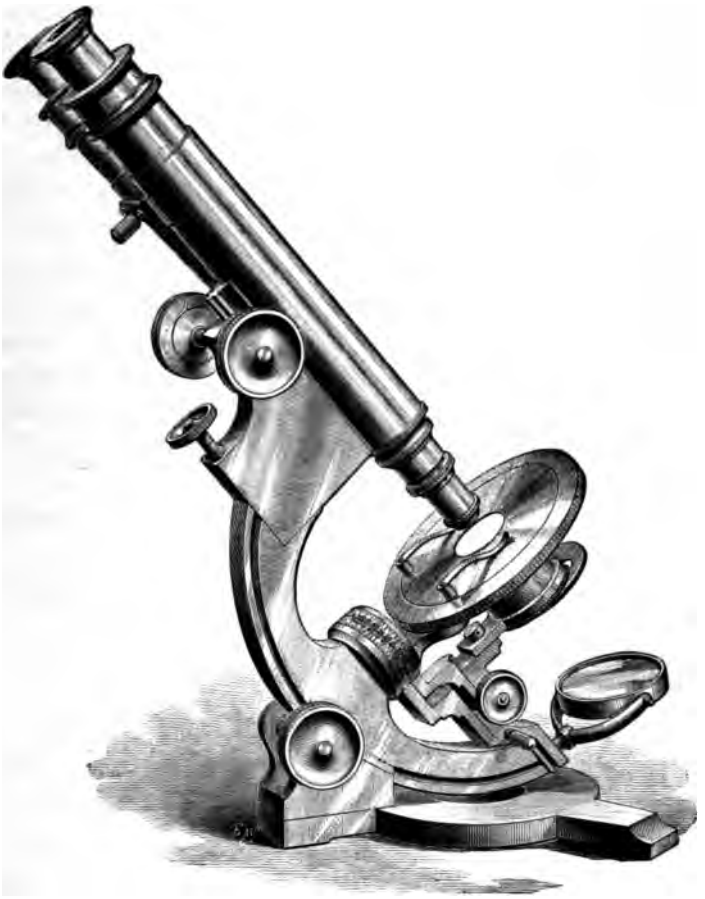


FIG. 31.

considering the fact that it is an innovation on the present mode of constructing microscopes, it may be said to have had a very favourable reception.

It is constructed entirely of brass. The base is large, of the tripod form, and has a circular well in the centre; the arm is a segment of a circle of which the centre of the stage is also the centre and is provided with two grooves; to these are fitted two fixed tongues and attached to the pillars; the grooves work on the tongues and thereby give the inclination of the body; the friction is just sufficient to retain the body at any point, but it may also be more firmly fixed by a tightening screw with large milled head; stops are provided for the arm in vertical or horizontal position. The stage revolves, and is $4\frac{1}{2}$ inches in diameter. The coarse adjustment is by rack and pinion of long range, the fine adjustment by micrometer screw, acting on a patent movement. The mirror and substage bars are separate, and swing together, or independent of one another, to any obliquity below or above the stage on a large bearing, the axis of which lies in the plane of the stage; both are graduated and silvered on their circular parts. The mirrors are plane and concave, of large size, and are adjustable on the mirror-bar; the substage is adjustable by rack and pinion, and is provided with a new contrivance (not shown in the cut), whereby it may be quickly swung to one side without varying its distance from the stage.

A new method of suspending the main limb of the microscope was devised some years ago by Mr. George Wale, U.S., which has lately been adopted into this country by Mr. Swift. Mr. Swift has written the author to say, that being so satisfied of the superiority of this method of suspension, he intends to give special prominence to this model, an illustration of which is shown in Fig. 32.

The stage and optical body are supported on a curved limb, having sectorial grooves on either side and slides between corresponding curved jaws on the inner side of the upright pieces of the foot. Mr. Swift has lately increased the

FIG. 32.

FIG. 33

length of the radial inclining limb, thus providing space for the complete rotation of the ordinary form of mechanical stage.

The fine adjustment is supplied to a slide in front of the coarse adjustment slide, so that the whole body tube is acted upon, and not merely the nose-piece, two adjusting screws

FIG. 31.

enabling the movement to be regulated with the utmost nicety. By this system of fine adjustment, the binocular prism is brought nearer to the posterior surface of the objective system, by more than a quarter of an inch, than in any microscope previously made by this firm. The coarse adjustment consists of the double stepped diagonal rackwork, suggested by Mr. J. Mayall, jun., and the tripod is so arranged that the crank arm of the mirror is allowed free lateral play, even when the instrument is placed in the vertical position.

Before leaving the subject of the microscope stand, it may be as well to give two illustrations of a model which has recently found favour in several medical schools and colleges on account of its low price. We refer to the microscopes of Herr Leitz, of Wetzlar, of which Fig. 33 is an illustration of the No. I. stand, designed with a view to render substage manipulation as simple and as perfect as possible. This instrument is very massive, and the stage firm and without tremor, but it does not seem to be very extensively used in this country.

The illustration shown in Fig. 34, is the instrument largely used in some biological classes, though, no doubt, for general purposes, the vertical position of the microscope is a very inconvenient one for the observer; still, there is no doubt that a large number of operations where fluids are dealt with necessitate the stage being maintained in the horizontal position, and for such purposes as these this stand is well adapted.

We cannot too earnestly exhort the young microscopist to endeavour to use the instrument with as much ease to himself as possible; observations should be discontinued whenever the eyes become fatigued, and this will always be found to take place sooner when the microscope is placed in the vertical position.

CHAPTER III.

BINOCULARS.

BINOCULAR microscopes are constructed so that the observer may use both eyes in the examination of an object; but whether a binocular instrument enables us to form a more correct idea of the form of an object is still a disputed point. It is true that for low powers there is often considerable advantage in the use of a binocular, but many accessories cannot be used with it, and, as it is usually constructed, the body tubes cannot be shortened, which is certainly a weak point. The stereoscopic effects of a good binocular render many objects more attractive to the eye; but persons whose means are limited need never regard this form of instrument to be indispensable in the production of good or accurate work, nevertheless we have always advised those to whom money is no object, to purchase a binocular, as continued observation is less tiresome and fatiguing when both eyes are employed.

Binocular instruments are constructed in such a manner that the rays of light proceeding from an object lying upon the stage are divided by means of a prism, and sent through a breeches tube, as shown in Fig. 35.

The form of prism used (we may say universally) is that devised by Mr. Wenham many years ago. It is usually

mounted in a small brass box, and slides into the end of the microscope body immediately over the objective as shown in Fig. 35. A section of this prism is shown in Fig. 36, the dotted line indicating one portion of the divided ray. The great advantage of this system is, that the instrument may instantly be converted into a monocular by simply withdrawing the prism.

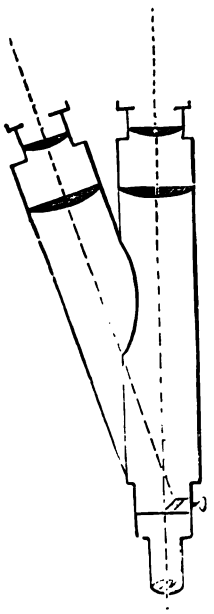


FIG. 35.

The Bausch and Lomb Optical Co. have lately introduced a very decided improvement in the method of mounting this prism, which is shown in Fig. 37. Instead of being mounted in a sliding box, it is fixed in a swinging carriage *b*, of which the axis is in *c*. The carriage *b* is fixed to the steel spindle *c*, which in turn passes through a sleeve in the nose-piece, and on the outside and in front of it is provided with a milled head, by which it is turned; the spindle and sleeve are arranged with stop-pin to limit the motion of the prism; *e* is the vertical, and *f* the oblique tube. As the posterior system of any objective with society screw does not exceed 15 m.m., the opening *d* in the nose-piece is made of this size. When the prism *a* is in position for binocular vision, all the rays coming from the objective will be utilized, whereas when the instrument is used as a monocular and the prism is swung to the side of the tube, as shown in dotted lines, the opening *d* is left without obstruction. In addition to this advantage, the fittings are all close, so that there is no opportunity for the dust to enter, and being cylindrical there is practically no wear.

It should not be forgotten that a binocular of this form is

not suited for use with objectives of higher numerical angle than 0·34 (40° air angle), a certain amount of distortion occurs with higher apertures, which may be readily perceived on the examination of spherical bodies.

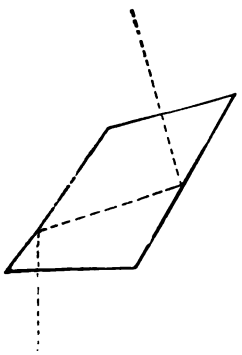


Fig. 36.

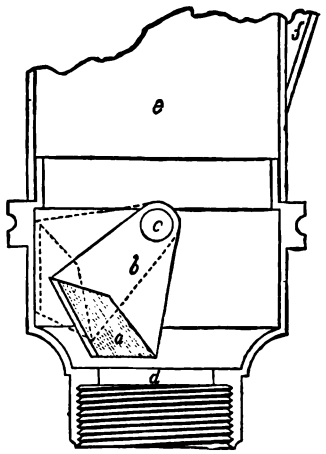


Fig. 37.

In order that this prism may be used with high power objectives, Mr. Wenham has devised means whereby it can be placed immediately behind, and nearly close up to, the posterior lens of the objective, but as a rather delicate adjustment is required this method of high power binocular vision can scarcely be considered satisfactory.

Mr. Wenham has devised another form of prism specially for use with high powers, as shown in Fig. 38. It is mounted in the same manner as the ordinary prism, for which it can be readily substituted, but the difficulties of construction are so great that we have never met with a good one, unless made by Mr. Wenham himself.

Messrs. Powell & Lealand also manufactured a binocular prism for use with high powers which answers perfectly even with the one-sixteenth objective. The prisms are mounted

in a box which can be readily substituted for the ordinary Wenham prism. It is shown in Fig. 39.

Another binocular microscope was devised some years ago by Mr. J. W. Stephenson, which has since been successfully

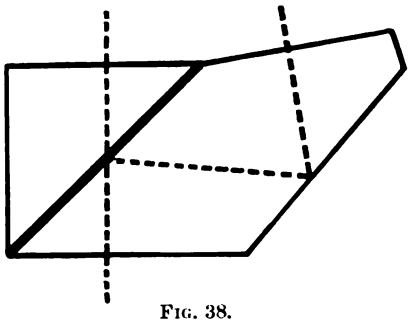


FIG. 38.

made by Mr. Browning. Within the last few years the cost of this instrument has been considerably reduced, Messrs. Swift and Son and Mr. Baker have each constructed a Stephenson binocular for dissecting purposes at the moderate price of £7.

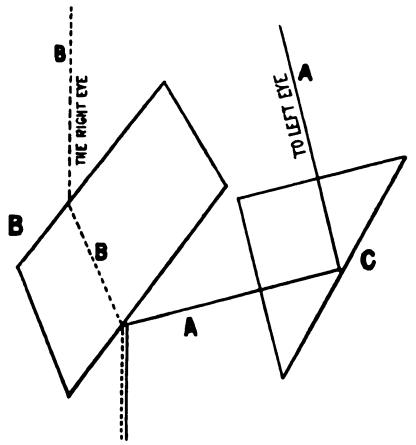


FIG. 39.

The Stephenson binocular, as made by Mr. Browning, is shown at Fig. 40, in which the change from binocular to

monocular or *vice versâ* can be effected without unscrewing any part or interfering with the object under examination.

In this form of binocular the rays are divided by two

Fig. 40.

prisms, A A, Fig. 41, which, passing upwards, are reflected towards the eye-pieces by the triangular prism B, Fig. 42. The instrument is erecting—that is to say, objects are presented to the eye in a normal manner, and not inverted as

in the ordinary form of instrument, which adapts it specially for use in dissecting. The bodies of the microscope are made to rotate, carrying the prisms with them, so that two observers may work with the instrument, observe the same object, and compare notes without altering the conditions under which the object is being examined.

M. Nachet, of Paris, has also exercised much ingenuity in the construction of binocular microscopes of special pattern, and several of them are worthy of more attention than has been bestowed upon them in this country. On the Continent, however, the binocular instrument is only found in the hands

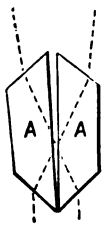


FIG. 41.

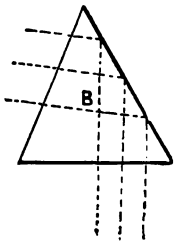


FIG. 42.

of a few, as a very strong opinion prevails—perhaps not without foundation—that for actual work the monocular is preferable.

In order that the single tube microscope may yield an image to both eyes simultaneously, the late Robert Tolles adapted a binocular eye-piece to the ordinary single body which gave a large and well illuminated field with the medium and higher powers. Professor H. L. Smith states that he has used the $\frac{1}{12}$ and $\frac{1}{16}$ objectives with it in a very satisfactory manner.

Carl Zeiss, of Jena, does not make binocular microscopes on the English pattern. All his tubes are monocular, but at

the same time, he supplies Abbe's binocular eye-piece, shown in Fig. 43, when binocular vision is needed.

As will be seen in the illustration, the box A, A′ contains three prisms of crown glass *a*, *b* and *b′*, the two eye-pieces are let into the top of the box, B being fixed, while B′ has a lateral sliding movement by means of the screw D to accom-

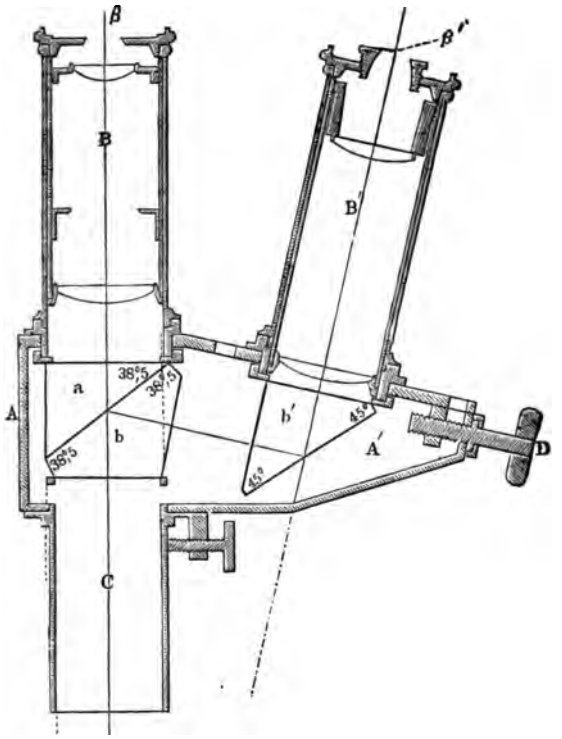


FIG. 43.

modate the distance apart, to the eyes of the observer. The two prisms *a* and *b* are united so as to form a thick plate with parallel sides, separated only by an exceedingly thin film

of air, the plane of the junction being inclined to the axis at an angle of 38°.5.

The cone of rays from the objective is split up into two portions, one being transmitted through the prisms *a*, *b*, while the other half is reflected through *b*, *b'* and upwards through the eye-piece in B'. The eye-pieces are furnished with caps, circular and semi-circular, and by the proper manipulation of these, either stereoscopic or pseudoscopic effects can be produced. Stereoscopic effects were at first produced by covering up the inner halves of the eye-pieces with the semi-circular caps, and pseudoscopic by covering the outer halves; but Professor Abbe has since pointed out that it is only necessary to cover up the half of one of the images, and if it be the lateral one which is half obscured very little loss of light occurs. The normal division of light in the two tubes is two-thirds in the axial and one-third in the lateral, so that if the lateral image is half obscured the loss of light is about one-sixth of the whole.

It will be as well to close this chapter by quoting a few remarks by Professor Abbe on the advantage of the binocular, published in the *Zeitschrift für Microscopie*, ii. p. 80. He writes:—

"There is an advantage to be gained from binocular vision which is quite independent of its stereoscopic effects, and is, in fact, of importance precisely to those observers who have least to expect from the latter. I have been told by many competent microscopists in England that they use their binoculars, whenever it is practicable, but not for the stereoscopic effect, but rather in order to employ both eyes, and so avoid the evil effects which the continual strain on one eye, in course of time, occasions. I have also found, from my own experience, that it would be well if regard were paid to this. It can scarcely be doubted that continuous one-sided vision, by those who use the microscope incessantly, must

gradually diminish the ability to use the eyes for ordinary vision—for example, in preparing objects and other work. From this point of view a binocular arrangement may be of value to those for whom stereoscopic observation is of subordinate interest."

CHAPTER IV.

THE HUMAN EYE.

IN all studies, whether of pure Microscopy as a Science, or whether of one of those departments of Natural History in which the microscope is employed as an aid to vision, we must, at the outset, recognise the importance of a study of the human eye. It may be the seat of many imperfections resulting from misuse, old age, or disease, which are apt to modify the conclusions we may draw from our observations, unless we are careful to study well into what lines such imperfections may lead us.

Nature has given us in this organ a means whereby all objects may be compared with each other, more especially as to size, colour, and general characters, and it must astonish the student, who thinks deeply, to find that so little is known definitely as to *how* we are able to appreciate magnitudes, colours, and forms. It is easy to say that the eye lenses focus a picture of the object upon the retina, and the irritations are carried by the optic nerve to the brain, but do we practically realize what this means? Then again, unless more than one of our senses are brought to bear upon a matter under consideration, we can scarcely form a true opinion upon our subject.

Take something which greets our vision for the first time. We know not what it is; we can see it, it is true, but we have to bring in the aid of other senses before we can arrive at a correct judgment; and even then, our judgment being the result of comparison, and also of experimental contact of substances with our senses—so to speak—opinions which are formed must, to a certain extent, be modified by the amount of other experience to which our nerve centres have been previously subjected. Take two experts; give to each one a sphere composed of lead and tin. Upon asking them what substance they were handling they might probably guess, perhaps not; they would poise it in their hands, look at it, smell it, try to cut it, perhaps, examine its metallic

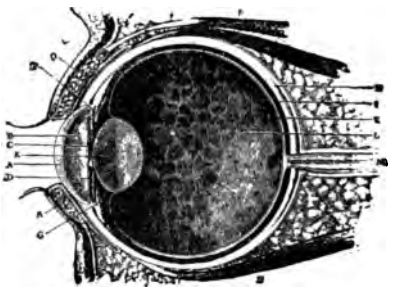


FIG. 44.

lustre, and it would be very odd indeed if they could agree as to the composition of the alloy, unless settled by an assay upon the balance. Has it ever occurred to the reader that such processes as these go on in Microscopy, and that it is necessary to carefully study the organ of vision in order to gain a true insight into the object presented to us?

On reference to Fig. 44, it will be seen that the eye is a nearly spherical ball, capable of many movements in its socket. It possesses an outer translucent covering called the sclerotic coat, or simply sclerotica, which may be seen at S. This is thick, horny, and opaque, except in its anterior portion.

This sclerotic coat envelops about five-sixths of the eyeball, and in common parlance is called the white of the eye.

The anterior transparent portion is called the cornea, and has the shape of a very convex watch glass. It is through this membrane that the light passes to the interior of the eye. The cornea and the interior portion of the sclerotica are covered with a mucous membrane.

Behind the cornea is a diaphragm of annular form called the iris; it is coloured and opaque, the circular aperture in its centre, *p* being called the pupil.

The iris, *i*, serves the purpose of regulating the admission of light; it varies in colour in different individuals, and is the part referred to when we speak of the colour of a person's eye.

Behind the pupil is the crystalline lens, *l*, having a much greater convexity at its posterior surface than at the anterior.

The large posterior chamber is lined by the choroid coat, and this choroid has in front of it a delicate membrane called the retina.

The choroid coat consists of a highly vascular membrane containing pigment cells, filled with an intense black mucus, called the *pigmentum nigrum*.

The cavity behind the cornea is filled with a liquid called the aqueous humour, having a refractive index approaching that of 1·3366, while the larger cavity is filled with a transparent jelly, called the vitreous humour, possessing a refractive index of 1·3379, enclosed in a very thin, transparent sac, called the hyaloid membrane.

Having now described the principal apparatus of the eye, we may take some of the parts in detail.

The crystalline lens is built up of layers, increasing in density inwards, the effect of which is to diminish spherical aberration. This lens is enclosed in a transparent capsule,

held in position by an elastic membrane. It can be changed in shape by means of a delicate muscular arrangement to adapt its focus for near or distant objects.

As glass lenses of varying curves have different focal lengths, so by altering the curves of the crystalline lens we are able to see objects distinctly which are situated in several focal planes.

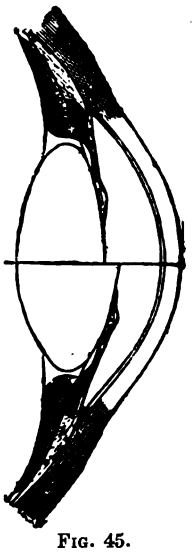


FIG. 45.

The reader may have noticed that there is a near point at which objects can be seen most distinctly; this point varies in individuals, but averages from 8 to 10 inches. As we move farther away from the object, although diminished in size, it may be seen more easily, and with less effort.

It would appear, then, that all objects are rendered apparently larger, as they continue to approach the eye, but a limit is soon found to this, as at a distance of six inches distinct and easy vision is not possible (except in very abnormal cases).

The reason of this is well known—the anterior focal point of a convex lens when shortened lengthens the posterior conjugate focus, so that when an object is brought too near the eye the image of it is projected behind the retina, and the crystalline lens cannot accommodate itself to such extremes. But we know that objects can be seen distinctly at great distances apart, and it may be useful to demonstrate how this is brought about.

The figure (45) represents a cross section of the crystalline lens. The real mechanism of accommodation has been much disputed, but the results, as observed, are, that the curvatures of the crystalline lens are altered as the observer adapts his

eye to near or remote vision; increase of curvature, of course, shortening the focal length of the crystalline lens, and being better adapted for near vision, while the shallower curve is necessary for the distant view of remote objects. Helmholtz has shown that the radius of curvature of the anterior surface of the crystalline lens may be varied by means of the muscular arrangement, from 6 to 10 millimetres.

Myopia, or Near-Sightedness.—The difficulty to see distant objects distinctly arises from a defectively constructed eye-ball, the distance from the lens to the retina at the back part of the eye being too great, and, as a consequence, the rays of light coming from a distant object are bent to a focus before reaching the retina (as shown in Fig. 46), and form on the retina only confused pictures.

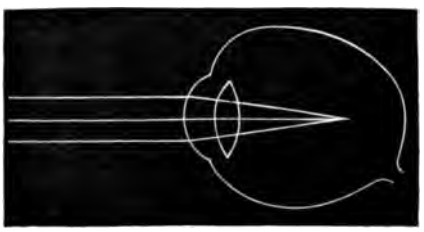


FIG. 46.

Myopia is a condition of the eyes existing at birth in most of the cases where it is discovered, but it may be contracted in youth by long continued application to reading, writing, or sewing, particularly where the light is poor and the head is held close to the work to overcome the want of light. It then may be due either to the failure of the muscle of accommodation to relax itself, or of the lens to regain its normal form.

Myopia, although transmitted from parents to children, is seldom noticed before the seventh year of age; but after that it begins to manifest itself in those whose antecedents

predispose them to it. But myopia is liable to be contracted by children of families where a near-sighted member was previously not known. It may then be the result of a prolonged and steady looking at an object, or at objects near the eye, though at proper distance, without rest or frequent change of the visual focus, as in long and absorbed novel reading, intense study, or persistent diligence in needle-work. The practice of reading or otherwise using the sight at too short range; this results in part from insufficient light, or from its faulty direction, so that the hand or body throws a shadow on the page, or the direct rays fall upon the eye, causing undue contraction of the pupil, while the page is in shadow. A prone or forward position of the head, too long maintained, or frequently repeated, and becoming a habit; this results from reading or studying with the book in the lap, and from the use of desks not graded to the height of the pupil. Donders says: "In the hygiene of myopia, the very first point is to guard against working in a stooping position."

Myopia is an accompaniment of civilisation, being, it is said, almost unknown among barbarous nations. It is rare among the poorer classes, and of these the inhabitants of cities, from the nature of their occupations, are more liable to it than inhabitants of the country.

Hypermetropia.—Hypermetropia arises from a defectively constructed eyeball, but it is the reverse of myopia, being too short from the lens to the retina, consequently the rays of light from distant objects are not sufficiently bent so that they will come to a focus on the retina, when the accommodation function of the eye is not exercised, as shown in Fig. 47.

The impressions of the object formed on the retina are then confused, very much the same as is the case with myopic eyes. Hypermetropia is also a birth inheritance, where it is found to exist in after years, though old-sighted people are apt to become hypermetropic after their sixtieth year.

Physicians who give their special attention to the eye and its defects, tell us the most frequent occurring cause of weak eyes is this defect called hypermetropia. Nature has made it possible, however, for persons having such eyes, to see distinctly by making their crystalline lens stronger—that is, shortening its focal length, so that the focus is brought forward on to the retina, and they see for the time being distinctly. This is done by means of the ciliary muscle, situated near the circumference of the lens within the eye-ball. In order to maintain distinct vision, this muscle of accommodation must be kept in a constant state of tension, under which it soon becomes exhausted, and gives up, or contracts spasmodically, at first causing blurred and indistinct vision, finally causing pain which may be felt in the eye, but

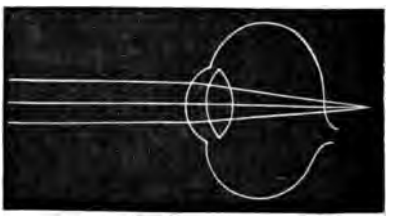


FIG. 47.

is oftener referred to the forehead and temples. This malformation is often the undiscovered cause of the headaches and neuralgias from which so many persons are constant or periodical sufferers. This, too, is one of the fruitful causes of the sick-headaches, etc., which are the penalty frequently paid for an evening spent at a place of amusement; the burning in the eyes, etc., being laid to bright gaslight, when in reality it was caused by the strain upon the little muscle within the eye in maintaining distinct vision.

We may now cast another glance at the iris. This apparatus is really a continuation of the choroid tunic which lies between the sclerotica and the retina: it ends in front, in

what are called ciliary processes, as shown in Fig. 48. The small muscular ring surrounding the pupil is called the sphincter muscle.

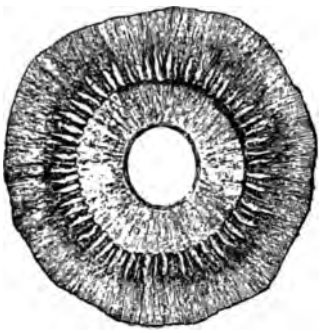


Fig. 48.

Now, the principal use of the choroid tunic, or rather the *pigmentum nigrum* which it contains, is to absorb those rays of light which have passed through the transparent retina, preventing their reflection, which would interfere with the distinctness of the image.

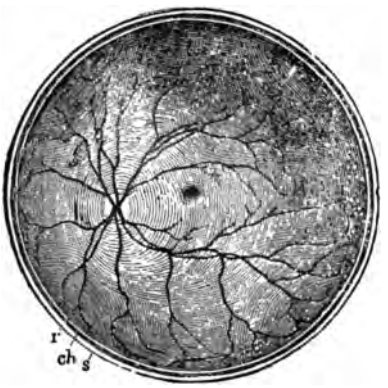
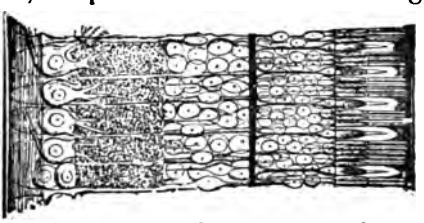


Fig. 49.

By referring to Fig. 49, after Henle, it will be seen that the choroid tunic, the retina, and sclerotica, form the three

outside rings, while the centre is ramified by nerve filaments and blood-vessels.

These nerve filaments and blood-vessels lie in the retina, which really forms a continuation and extension of the optic nerve; it touches the outer circumference of the iris at the front, and lies open as a cup-shaped disc in the interior of the eye; it receives the rays of light which have passed in turn through the cornea, aqueous humour, crystalline lens, and vitreous humour, and forms a picture at the focus of these. The nerve fibres of the retina are excited probably by a product of the action of the light picture upon the visual purple, and the irritations are transmitted to the brain by the optic nerve, producing the sensation of vision. The picture produced upon the retina has been compared with that produced by a photographic lens upon the screen or ground glass; but it will be seen that the instances are not strictly parallel. In the eye the rays falling upon the cornea do not again encounter air, the picture is formed *in* the highly refractive



a. b. c. d. e. f. g. h. i.

FIG. 50.

substance, while in the photographic image air intervenes between the screen and the lens, and between the lenses themselves. Then, again, the adaptation of the eye to various distances is obtained by a process so dissimilar to that of the lens in the camera, that it is well no comparison should be instituted. The retina has been previously described as a delicate membrane lining the choroid tunic, inside the sclerotica.

Now, if we make a section of the retina, we shall probably find its structure very similar to the diagram (Fig. 50). It is well to say probably, as the author has never met with sections displaying the structure so well as Max Schultze has indicated. He has described the various layers which you see before you, as follows:—

Starting from the junction of the retina with the vitreous humour, we have—

The layer of nerve fibres.....*a*.	The outer granular layer .....*f*
The layer of nerve cells.....*b*.	A second fine membrane.....*g*.
The granular layer.....*c*.	The layer of rods and cones.....*h*.
The inner granular layer.....*d*.	Pigmentum nigrum of the choroid.*i*.
The intermediate layer.....*e*.	

The retina is the terminal organ of vision, all the apparatus in front of it being merely for the purpose of securing that an accurate image shall be focussed upon it. As to how the luminous impressions yield to us such a definite idea of things is a question still under consideration, many have tried to solve it, but it is not certain that we are any nearer the mark than those philosophers were who lived 2,000 years ago.

There are several curious properties inherent in the retina. By means of the opthalmoscope may be seen a point, a little out of the centre, where the optic nerve enters the eye. This spot is totally blind, it cannot perceive a trace of light, and if the image of an object falls upon this blind spot, that object is totally invisible. It is at this spot also where the blood-vessels enter the eye, and ramify through nearly the whole of the surface layers of the retina.

In the centre of the figure (49) you will see also a dark shaded portion practically free from blood-vessels. It is a round, yellowish, elevated spot, about $\frac{1}{24}$th of an inch in diameter, and it is here that the sense of vision is most perfect. It is called the yellow spot of Sœmmering; it is not covered by the

fibrous part of the retina, but a layer of closely-set cells passes over it, and in its centre is a minute depression called the *fovea centralis*.

In the above description, points only have been touched which directly bear on good or defective vision. On the other hand, enough has been advanced to show that this organ is liable to imperfections which may, and are extremely liable to, modify all our observations made over the tube of the microscope.

In order to produce a picture upon a screen, a lens is not absolutely necessary; if a diaphragm, perforated with a series of small holes, be placed in front of the electric lamp, the screen will be decorated with as many images of the carbons as there were holes in the diaphragm; but another illustration will perhaps render this more evident. A small hole pierced in the shutter of a darkened room (Fig. 51) allows of

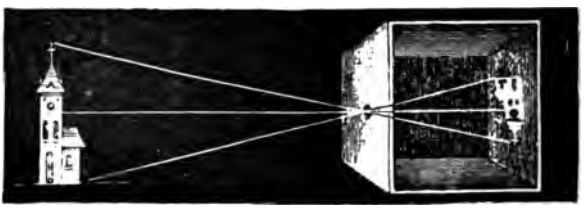


FIG. 51.

the passage of rays from a well-illuminated landscape, so that a small but inverted image is cast upon the screen; the further the screen is placed away from the aperture the larger will the image be, though less distinct, and *vice versa*. The picture produced is not so good as that formed by a lens, it is dark and somewhat confused at the margin, and if the aperture is enlarged, there is still greater confusion, until the image is finally lost.

Now, if we take an ordinary lens of glass and attempt to produce a picture with it, we find the centre alone is plainly

visible—the lens is afflicted with what is termed *spherical aberration*, that is, the rays from its periphery are brought to a focus in a different *plane* to those occupying a central position, this fault may be illustrated by Fig. 52.

But although it is so easily shown in a diagram, a small amount of spherical aberration is not so easily detected by the student. It appears as a haze or fog of light over the object.

In the human eye this defect is not observable to any great degree, as the peripheral or more strongly refracting rays are cut off by the iris. Then, again, the curvature of the cornea is ellipsoidal rather than circular, so that the rays farthest from the axis are least deviated, while the two curves of the crystalline lens correct, so to speak, the one the other; and lastly, this lens is of such construction that its refractive power diminishes from the centre to the circumference.

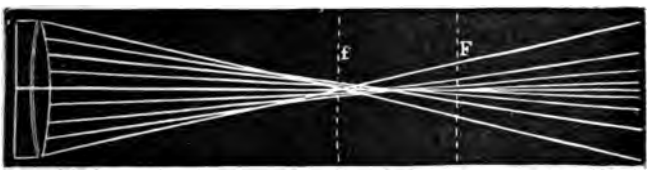


Fig. 52.

Another defect in the eye is due to the different meridians having dissimilar degrees of curvature.

This defect is called astigmatism, and known to oculists as a common cause of headaches. Spasms of the focussing apparatus may derange the sphericity of the eye, and so affect vision. Strained vision is very subject to this. On the other hand, the same apparatus may be paralysed, and ordinary vision deficient, whilst the focussing of the microscope might possibly correct it.

In the perfect eye, the cornea is nearly the segment of a sphere, and the pupil is about opposite the centre or apex of

that sphere. But it frequently happens that the cornea is not the segment of a sphere, but is more convex in one direction than in another—in other words, of unequal curvatures in directions at right angles to each other. The consequence of this irregularity upon the sight, is, that while the eye may be perfect for seeing objects that are perpendicular, it is defective at the same time for seeing horizontal objects, or *vice versa*. An astigmatic person generally holds objects close to the eyes in order to enlarge them, and so in a measure compensate for the loss of sharpness of vision. When looking at a series of parallel lines like those illustrated in Fig. 53, an astigmatic eye will

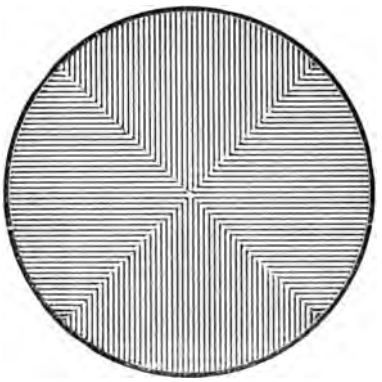


FIG. 53.

often only be able to see one set clearly, those in the reverse direction being blurred and indistinct. People having a high degree of astigmatism frequently consider themselves near-sighted, and are surprised, when attempting to obtain glasses, than none can be found among concave glasses that will greatly improve their vision. Children who learn to read with difficulty, annoying their parents and teachers by their apparent stupidity, are generally troubled with astigmatism, which can only be remedied by cylindrical glasses, carefully ground to the oculist's order after accurate measurement of the defect.

The eye may be myopic and astigmatic at the same time, or hypermetropic and astigmatic, or simply astigmatic. To correct astigmatism of the eye and render good and comfortable sight possible, a lens must be selected one surface of which is the segment of a cylinder; this, by its concavity or convexity, and placed in proper position, will correct the defective curve of the cornea, without interfering with the more perfect curve of that cornea.

Astigmatism has injuriously affected painters; Turner for instance, whose later pictures have been discovered to be

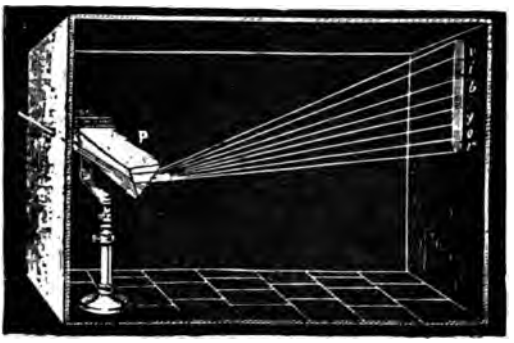


Fig. 54.

slightly distorted, in consequence of the power of accommodation or self-correction having been lost from age.

In microscopic drawing, as with the camera lucida, the perspective may be misrepresented, in consequence of astigmatism, and thus endless disputes may arise even among the most careful observers.

We have now to deal with errors of refrangibility, and it will probably have been assumed that the eye apparatus is entirely corrected for colour. This is not the case, however, except when an object is in exact focus, and the reason that the error due to refrangibility remains practically unnoticed is that the distance between the focal point of the red and violet

rays is extremely small. The error due to refrangibility may be noticed by means of the concentric circles already referred to; by bright daylight adjust the eyes to some object twelve inches away, and without moving the eye insert at a distance of four inches a card inscribed with black circles, when a yellow and blue colouring will be plainly discerned.

In order that the reader may thoroughly understand the error of refrangibility, the picture afforded by the passage of a solar ray through a prism of glass may be thrown upon a screen; the rays are deflected unequally, the red least and the violet most, as in Fig. 54.

It may be advisable here to state that the degree of dis-

FIG. 55.

persion of the rays of white light depends upon the medium through which the ray passes, and this amount of dispersion is measured by the distance of the most prominent dark lines in the spectrum from each other. The diamond disperses much less than crown glass, while the deflection of the ray is greater; but this is a subject beyond the scope of the present chapter.

Now, beside these errors, there are others to which the microscopist should devote special attention; they are caused

by small opaque particles existing in the transparent media of the eye-ball. These cast their shadow on the retina, and produce images which appear to exist outside the eye. These extra-retinal images often appear as globules, bacterioid-shaped bodies, or strings of minute pearls, and may be studied by directing the eye to a sheet of strongly illuminated opal glass, through a small aperture made with a fine needle in a piece of thin blackened cardboard. (Fig. 55.)

When the microscope is used in a vertical position, these globules often gravitate to the centre of the cornea, and even after prolonged use of the inclined tube an observer may often be perplexed by the layer of mucus, or a lachrymal discharge covering the surface of this organ.

Just a few words as to colour perception. Colour is a special sensation excited in the retina by rays of a definite wave length, and the reason why certain objects are presented to our view with colour is that when white light falls upon a given surface, some is absorbed, the remainder being reflected. If the green rays are reflected, then the object appears green, and if the red rays are alone reflected, then the object will be red.

The generally accepted theory of colour perception is based on the assumption that three kinds of nerve fibres exist in the retina, the excitation of which produces sensations of red, green, and violet, and that modifications of these three sensations yield all intermediate tints.

This theory will explain some of the phenomena of colour blindness—if the nerve fibres which should give their special sensation are paralysed, or are wanting, the sensation only of the complementary tint will be transmitted with all the defects of the eye. It must not be forgotten that many phenomena consist more in errors of judgment than in absolute error of form or sensation.

Now in regard to errors of judgment, we must admit that

all our estimations are made by comparison. In magnitude we are guided by the size of the retinal image as determined by the visual angle—for position we must have some starting point; and as for distance, everyone knows how delusive an inexperienced estimate of this is. At sea, a landsman could not judge of the distance of a passing vessel to a few miles, nor could we form any accurate idea of the size of any object emitting practically parallel rays unless we had something to compare it with.

We now come to a point which has been much disputed in the study of microscopy—binocular vision.

The two eyes move together as a system, so that we direct the two lines of regard to the same point in space and consequently see but a single image; but it is possible to see two. If one eye be displaced a little with the finger two images are seen, while if the other be displaced to a corresponding degree the one image is restored.

The value of binocular vision may be easily ascertained by experiment. When a picture is presented to the retina of each eye, the compound picture is much brighter than when one retina only is employed. To each point of the retina of one eye there is a corresponding point in the retina of the other, and impressions produced on one of these points are in ordinary circumstances indistinguishable from a similar impression produced on the other. When both retinæ are similarly impressed, the general effect is that the impressions are more intense than when one eye only is employed; and we also get a perception of relief, that is of form in its three dimensions.

Take two A eye-pieces and look through them to the sky, so that two distinct circles are seen; now bring them together so that one circle overlaps the other, when this overlapping bi-convex portion will be found double the brightness of the remaining portions of the circles. Binocular vision should be

employed wherever practicable; it will be found much less trying to the eyes than monocular efforts.

We are indebted to stereoscopic vision for the perception of relief or form in three dimensions, which occurs when the images falling upon the corresponding points of the two retinæ are not exactly similar. In looking at an object with both eyes the rays do not run parallel from one side of the object to the eye on that side, but the right eye centres itself to the left side of the object and *vice versâ*. This may readily be seen by holding up a finger between our eyes and the wall, and looking at the latter. Two fingers may be seen projected

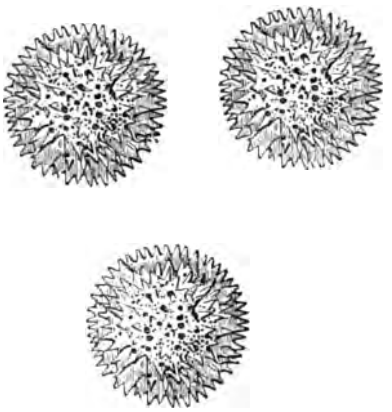


Fig. 56.

on the wall, one of these is seen by the right eye and the other by the left; but our visual impressions do not inform us which picture is formed by either eye in particular. Now, while steadfastly looking at the wall, close the right eye and the left finger will disappear, while on shutting the left eye, the right finger is rendered invisible. When two similar pictures are presented to the eyes, the impression is more vigorous and looked at with greater ease than when one eye only is employed; vision in this case is called pseudoscopic.

It may now be seen that the human eye is extremely liable to imperfection, and, being so, strict attention to details is demanded from the microscopist.

Although the human eye is such a wonderful instrument, there are many problems it is unable to solve without extraneous help. Take, for example, the pollen of the *Althea rosæ* (Fig. 56). With the unaided eye, nothing more can be discerned than a yellow dust, the various details having to be made out by other means. Then, again, with objects so minute as the diatom, *Amphipleura pellucida* (Fig. 57). The object itself is almost invisible to the unassisted eye, to say nothing of the carvings with which the valves are embellished, and which exact for their elucidation the most perfect lenses with which we are acquainted, and the most accurate manipulation of the illumination. The contour of many forms of diatoms may be seen, it is true, without extra optical assistance than that afforded us by nature, but not much more than this, as, if the eye is approached too closely, the picture falls behind the retina and is lost.

Fig. 57.

It has been already mentioned that starting with the distance of most distinct vision, continued approach to the eye finally renders the object invisible, the rays being thrown *behind* the retina, the mechanism of accommodation being insufficient to produce a curve deep enough to bring the picture to a short conjugate focus. This can, however, be done by interposing a lens or lenses between the object and the cornea, so that an image of the object is seen. These lenses form either a simple, or a compound microscope.

CHAPTER V.

EYE-PIECES AND OBJECTIVES.

EYE-PIECES OR OCULARS. — When the student purchases a microscope stand, he will generally find it supplied with the lowest power Huyghenian or negative eye-piece, usually designated by the letter A. At the same time, it may be stated that others, possessing greater degrees of amplification are often substituted or added at the wish of the purchaser; and it should be remembered, in the selection of a microscope stand, that the eye-pieces of one maker will not always fit the tubes made by another. It is a thousand pities opticians have not yet learned that their time may be more profitably occupied than by making adapters for each other's instruments.

We have carefully measured the tube diameters of the microscopes of most of our leading opticians, and the dimensions may be found in the table on another page.

It often happens that the size of the ocular requires to be sent by post to a distance. This size is best obtained by gumming a strip of paper over the body tube and cutting it open when dry with a sharp penknife, or by cutting a strip of paper of such a length as will just meet round the ocular.

The Huyghenian eye-pieces or "oculars" of low power are generally styled "shallow," to distinguish them from

those which give greater amplification, which are called "deep"—the terms deep and shallow being applied to the degree of curvature possessed by the lenses employed in their construction, and not to the distance between them, as some writers have imagined.

A full-size section of the Huyghenian A eye-piece is shown in Fig. 58, so that the student may understand the details of its construction.

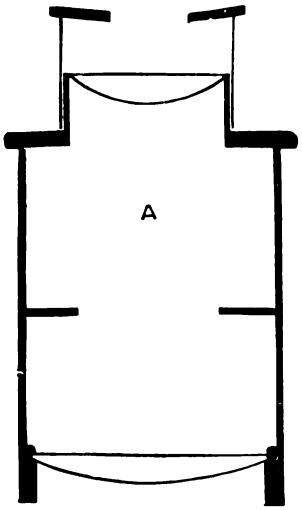


Fig. 58.

It consists of two plano-convex lenses, placed at a distance from each other equal to half the sum of their focal lengths, the best proportion of relative radii being 1 to 3.

The lower lens is called the field-glass, and the upper one the eye-glass, while a circular stop or diaphragm is placed nearly midway between the two.

The practical optician Gundlach has stated that the correction afforded by the Huyghenian eye-piece is not a complete one; for at the point where the spherical aberration is entirely corrected, the chromatic error has not completely disappeared: this even at the most favourable interval between the two lenses.

This eye-piece was first employed by Huyghens for his telescopes, in order to diminish spherical aberration and to increase the size of the field. An elaborate dissertation upon it has been published by Mr. Varley in the "Transactions of the Society of Arts," vol. li., to which the student is referred. It is often called the negative eye-piece, on account of its correcting the positive aberrations of the objective.

In Fig. 59 is shown a section of a deep eye-piece—the Huyghenian C—in which it will be seen that the lenses possess deeper curvature than in the A, while the diaphragm is more contracted, and the aperture in the cap covering the eye-glass is very small indeed; the C eye-piece gives about double the amplification of the A. There is another kind of ocular in occasional use, called Ramsden's positive eye-piece; it is formed of two plano-convex lenses, but the curvature of the field-glass is turned towards the eye instead of towards the object, as in the Huyghenian. In this eye-piece the focus is obtained *in front* of the field-glass, while in the Huyghenian ocular the image is formed *at the diaphragm*, about midway between the field-lens and eye-glass.

The Ramsden eye-piece was much in use at one time for purposes of micrometry, as it gave an excellent view of the

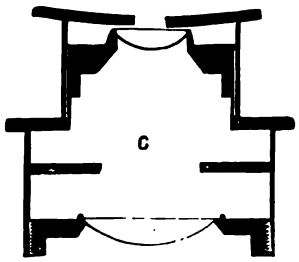


Fig. 59.

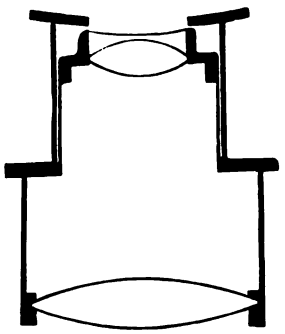


Fig. 60.

micrometer, free from distortion even to the edges of the field, though the image was slightly coloured. It is still used by Messrs. Ross and Co. for their eye-piece micrometers.

Kelner's orthoscopic eye-piece is much employed where a large and flat field is required for use with low powers. A section is shown in Fig. 60, from which the student may gather that the field-glass is doubly convex, and the eye-glass

a slightly under-corrected achromatic combination, while the diaphragm is dispensed with altogether.

In the ordinary or Huyghenian ocular, English opticians designate their power by means of letters, A, B, C, D, E, and F, while some few call their productions by the numbers, 1, 2, 3, 4, and 5; they seem fairly agreed as to what should be the relative degrees of amplification of the A and B eye-pieces, and some with the A, B, and C; but with higher powers there seems no uniformity, as the following table will show. The numbers have been calculated from the catalogues of the different makers:—

Oculars.	A	B	C	D	E	F
Ross	1·0	1·6	2·5	4·0	5·0	8·0
Powell and Lealand	1·0[1]	1·5[2]	2·0[3]	4·0[4]	6·0[5]	
Beck	1·0	1·6	2·6	3·2	5·2	
Swift	1·0	1·4	2·0	2·8	4·3	5·6
Browning	1·0	1·3	2·0	3·7		
Watson	1·0	1·4	2·0	2·5	3·4	4·3
Crouch	1·0[1]	2·0[2]	3·3[3]			
Collins	1·0	1·6	2·5	4·0		
Baker	1·0	1·6	3·0	4·5		
Pillischer...	1·0[1]	1·6[2]	3·0[3]	3·5[4]		
Parkes	1·0	1·5	2·2			
Zeiss...	1·0[1]	1·3[2]	1·8[3]	2·6[4]	3·5[5]	
Hartnack	1·0[1]	1·2[2]	1·5[3]	2·3[4]	3·0[5]	3·5[6]

In America oculars are treated in a more rational manner, they are styled as "2-inch," "1-inch," or otherwise, as the case may be, according to the degree of amplification they yield when compared with single lenses; thus a "2-inch ocular" would amplify the same as a single lens of 2-inch focus, and so on in like proportion.

Now reference to Chapter I. will show us that a single lens of 2-inch focus (equal to the English A eye-piece) magnifies about five diameters at a distance of 12½ inches from the micrometer to the screen, and the 1-inch, 10 diameters (equal to the C eye-piece); so that it is well the

student should as early as possible grasp the fact that the amplification of an object is arrived at by two stages, the objective producing an enlarged image of the object, which the ocular magnifies still further. Roughly it may be stated that if the inch objective be used with the 1-inch ocular, an amplification of 100 diameters is arrived at, the objective magnifying the object 10 diameters, the image of which is further magnified 10 diameters by the ocular; and further, if the same objective be used with the 2-inch ocular (the A eye-piece), the former will produce an enlarged image of 10 diameters, which the ocular will again magnify five times, producing a total amplification of fifty.

This is not mathematically exact without every disturbing element be taken into consideration, but is quite near enough to illustrate the case in practice. Fifty diameters is the recognised amplification for the 1-inch objective combined with the A ocular at a distance of 10 inches, and as the enlargement of the object takes place in two distinct stages, it will be seen that the optician is able to vary the powers of both ocular and objective and still obtain the standard result. An inch (so-called) objective magnifying 8·3 diameters when used with an A eye-piece magnifying the image six diameters, will give a normal result, as will an objective of the same designation magnifying 12·5 diameters with an A ocular magnifying four.

Similar cases to both of these have recently fallen under the notice of the author, and it is on account of like departures, that abnormal amplifications are obtained when the objectives of one maker are used with the oculars of another.

It often happens with new oculars that particles of brass get detached, and fall upon the inner surface of the lenses, which must be removed by unscrewing, and then *carefully* wiping with a *very soft* wash-leather. Dust specks and bubbles may be easily detected by deflecting a dull light

through the body of the instrument, when, by observation during the rotation of the eye-piece, they show very plainly. A good eye-piece should be perfectly transparent, and free from striæ and markings and spots of any kind. The marginal circle of the field of vision should be sharp, clear, and intensely black. If these conditions are not fulfilled, the eye-piece cannot be considered as perfect, or fit for general use.

Periscopic eye-pieces consist of a triple eye lens and a doubly convex field lens; they give an extremely large field, the image being sharply defined, even to the extreme edge. The 2-inch, or A, is of rather too large a size for the ordinary microscope; but the 1-inch, and ½-inch are useful sizes. They are positive, the diaphragm being placed below the field lens, and are therefore admirably adapted for micrometer work. They also give very good results as substage condensers.

For some time past, what are termed "Solid eye-pieces," have been manufactured in the States; they consist of one piece of homogeneous glass, ground to the proper curves. They are generally fixed in a standard size mounting, which is made to slide into an adapter for the body tube of the microscope. The most useful powers are ½, ⅓, and ¼ of an inch. The author has a very good $\frac{1}{8}$ solid ocular, made for him by the late Robert Tolles, specially for testing objectives, but for ordinary work it is of but little use, owing to the small amount of light it allows to pass.

We have always advised students to commence with the 2-inch ocular (A); if another is desired, the 1-inch (C) will be found of the greatest use, and choice may lie with the ordinary Huyghenian, the periscopic or orthoscopic C, or the Ramsden micrometer eye-piece.

The beginner is often much troubled, when commencing to use the microscope (the monocular), as to what he shall do

with the eye which is not over the tube. He should endeavour, as soon as possible, to get into the way of making his observations *with both eyes open*, and by this means he will find far less fatigue than when the one eye is closed. Some observers, however, even after years of practice, cannot perform their operations under these conditions, and therefore such an appliance as Pennock's eye shade (Fig. 61), made by Messrs. J. W. Queen & Co., of Philadelphia, is an important adjunct. It is made of blackened brass, to fit the neck of any ocular, and is so arranged that the blackened circular disc can either be used on the right side or the left of the observing tube.

FIG. 61.

OBJECT-GLASSES OR OBJECTIVES.

The history of the achromatic objective is a curious one —interesting certainly, but it should teach us the serious lesson not to be dogmatic in our assertions. Biot and Wollaston, the latter especially, were wedded to doublets, and they both predicted, on the faith of certain experiments, which were then unsuccessful, that the compound microscope would never excel the simple. How far this prediction has been verified most of our readers will know; but it is certain that Wollaston never thought that within fifty

years of his prediction the doublet would be a thing of the past, rarely heard of and never seen.

Light seems to have dawned upon objective construction through the elder Dollond, who employed two different kinds of glass in the construction of his telescopes. Recognising this principle, several foreign opticians made partly corrected glasses as early as 1824, and at the same time Tulley, of London, produced the first achromatic objective made in England; it was composed of three lenses, and possessed an air angle of 18°, which he soon after increased to 38° by placing another corrected combination in front of it.

In the year 1829, Mr. Joseph Jackson Lister, in his celebrated paper, published in the "Philosophical Transactions of the Royal Society," pointed out how many of the difficulties could be overcome, and exhibited an objective of 50° air angle which gave a large field and a correct image. This advance was so great that it astonished Dr. Goring, who wrote, in his "Exordium to Microscopic Illustrations," that "microscopes are now placed completely on a level with telescopes and, like them, must remain stationary in their construction."

Improvements, however, continued to be effected; Mr. Thomas Ross, upon increasing the air angle, discovered that different thicknesses of covering glass disturbed the corrections for spherical and chromatic aberrations, no matter how carefully made, and in 1837 he presented a paper to the Society of Arts upon the subject. In this paper he stated having made an improved combination, the focal length being one-eighth of an inch, with an air angle of 60°. After this he announced obtaining an air angle of 135°, and falling into a similar error of dogmatism as Goring, Wollaston, and Biot, stated that "135° is the largest angular pencil that can be passed through a microscope object-glass."

In 1851, Chas. A. Spencer, of Canastota, N.Y., produced objectives of 146° air angle, and in 1857 he constructed a one-twelfth with an air angle of 178°. Since this, Mr. Tolles, of Boston, has made lenses infinitely near 180°, and this angle in air has been approached by several English makers.

But these are not the whole of the improvements which have been effected; air lenses, or dry objectives, have been supplemented by water-immersion powers, and finally we have the homogeneous-immersion system, in which the transmitted ray pursues a rectilinear course from the under side of the object slide until it leaves the posterior surface of the front lens.

Objectives are generally spoken of in terms of the amplification which they yield, the standard of comparison being the magnification given by a single lens of the nominal focus. The student must not, therefore, imagine that an objective stated to be 1-inch, ½-inch, or so on, will focus at these distances from the object. Opticians have never used the term in that sense, though a few writers in public journals seem to have understood the nomenclature in that light.

LINEAR MAGNIFYING POWERS OF OBJECTIVES AND EYE-PIECES.

(WITH TUBE OF STANDARD LENGTH.)

	Objectives.	4 in.	3 in.	2 in.	1 in.	¾	½	1/10	¼	⅕	⅙	⅛	1/10	1/12	1/16
Eye-Pieces.	2 inch (A)	12	18	25	46	50	92	130	210	275	325	400	550	650	800
	1½ inch (B)	15	23	30	54	70	110	160	250	325	390	490	650	775	980
	1 inch (C)	23	30	45	80	90	165	240	375	485	580	750	970	1160	1500
	¾ inch (D)	30	45	60	108	140	220	320	500	650	780	980	1300	1550	1960

As to magnifying power, the foregoing list has been extracted from the catalogue of the Bausch and Lomb Optical Company, and will serve to show what degree of amplification is yielded by the various objectives, in combination with different oculars.

It may be useful also to add a list of the magnifying power of the oculars and objectives of Hartnack (now Prazmowski) and of Zeiss. They are used in this country to some extent, and the users always have the foible of omitting the numbers of diameters to which the object has been magnified, adding, however, the numbers of both ocular and objective, to which the student generally has no reference. It should not be forgotten that the amplifying powers of the foreign objectives are calculated for a tube of 153 millimetres (6 inches) in length, and most of these objectives lose in their performance if employed in the ordinary English length of tube.

Prazmowski (Hartnack) gives the following values for his oculars and objectives:—

Objectives.	Oculars.						Equivalent Focus in Inches.
	1	2	3	4	5	6	
1	15	20	25	...	...	...	2
2	25	30	45	...	...	...	1
3	50	60	80	120	...	...	$\frac{3}{4}$
4	60	70	90	140	...	...	$\frac{1}{2}$
5	100	125	160	240	...	...	$\frac{1}{4}$
6	150	180	240	350	...	...	$\frac{1}{6}$
7	200	240	300	450	600	700	$\frac{1}{8}$
8	250	300	400	600	800	1000	$\frac{1}{9}$
9	350	400	550	860	1100	1400	$\frac{1}{11}$
Immersion Objectives with Correction.							
9	410	480	630	950	1300	1500	$\frac{1}{12}$
10	520	600	750	1100	1500	1800	$\frac{1}{15}$
13	820	950	1170	1700	2370	3100	$\frac{1}{25}$
15	1040	1200	1500	2200	3000	3600	$\frac{1}{30}$
18	1560	1800	2250	3300	4500	5400	$\frac{1}{50}$

Zeiss, whose objectives have been much in request during the past few years, is noted for great working distance; his angles are low and he designates his objectives by letters, but gives also the equivalent focal lengths in inches and millimetres; they are as follows, the magnifying powers being expressed for a tube of 155 millimetres ($6\frac{1}{8}$ inches) in length :—

	Mark.	Equivalent Focal Length.		Air Angle.	Oculars.				
					1	2	3	4	5
		inch.	mm.	°					
Dry Objectives.	aa	1	25	20	20	27	36	52	70
	A	$\frac{3}{5}$	15	24	40	55	75	105	140
	AA	$\frac{3}{5}$	15	36					
	B	$\frac{2}{5}$	10	34	70	100	135	180	240
	BB	$\frac{2}{5}$	10	60					
	C	$\frac{1}{4}$	6·5	50	110	145	195	260	370
	CC	$\frac{1}{4}$	6·5	90					
	D	$\frac{1}{6}$	4·2	75	175	235	320	440	600
	DD	$\frac{1}{6}$	4·2	110					
	E	$\frac{1}{9}$	2·8	116	260	350	480	660	900
	F	$\frac{1}{12}$	1·8	116	410	550	750	1020	1390
Water Immersion Objectives.	G	$\frac{1}{8}$	3·0	1·15-1 17 or 108° water angle	250	340	450	620	840
	H	$\frac{1}{11}$	2·3		320	440	590	800	1100
	J	$\frac{1}{15}$	1·7		440	590	800	1090	1500
	K	$\frac{1}{20}$	1·3		590	790	1060	1450	1980
	L	$\frac{1}{25}$	1·0		760	1030	1380	1890	2580
	M	$\frac{1}{33}$	0·75		1010	1360	1840	2520	3450
Homo. Immersion.		$\frac{1}{8}$	3·0	1·25-1·30 or 116° Balsam angle	250	340	450	620	840
		$\frac{1}{12}$	2·0		390	520	700	950	1300
		$\frac{1}{18}$	1·3		590	790	1060	1450	1980
Equivalent focus of oculars ... mm.					48	42	30	24	18
Do. do. in inches					1·88	1·64	1·18	·94	·70

Herr Leitz, of Wetzlar, is now constructing some very fair and exceedingly cheap lenses which are largely in use in England. The amplifications of these objectives, when

used in a tube 160 millimetres long, equal to 250 millimetres visual distance are as follows :—

Focus.		Air Angle.	Amplifying Power.					
In.	MM.		O.	I.	II.	III.	IV.	V.
2½	58·4	10°	20	30	40	50	60	80
1½	30·5	20°	30	45	55	60	80	100
½	12·7	40°	50	80	90	120	130	145
⅜	7·6	60°	70	100	120	180	200	240
¼	6·4	80°	120	180	200	250	300	350
⅕	5·1	100°	180	260	300	255	450	500
⅙	4·2	105°	300	350	400	500	600	700
⅛	3·2	110°	350	500	550	650	800	1000
1/12	1·8	110°	500	700	800	1000	1200	1400
Water Immersion.								
1/12	2·0	Water Angle 112°	550	800	900	1000	1200	1500
1/15	1·5		700	900	1200	1500	1650	1800
1/18	1·0		900	1200	1500	1700	2000	2400
1/20	0·8		1000	1400	2100	2400	2600	3000
Homogeneous Immersion.								
1/12	2·0	Balsam Angle 116°	550	800	900	1000	1200	1500
1/15	1·5		700	900	1200	1500	1650	1800
1/18	1·3		900	1200	1500	1700	2000	2400

Objectives—which, by-the-by, are sometimes called "powers"—being made from glass of varying density and also of varying refractive indices, it follows that they must differ also in construction in some degree. The various lenses of which each combination is constructed are ground to a series of curves, suitable to the glass employed, and the combinations are placed at different distances apart, so that we can only give a rough outline of their general construction.

As a rule, objective mounts are turned out much too long. There is no apparent reason why the brasswork (of some

opticians especially) should not be considerably reduced. When the posterior lens is too far away from the Wenham prism, in a binocular instrument, it is extremely difficult to procure an equal illumination of the whole field—in fact, the general performance of the instrument is seriously interfered with, and therefore, for use with the binocular, short mounts should be preferred and the longer ones rejected.

Objectives are now-a-days made for use upon two different systems, dry and immersion. A "dry" lens is the one no doubt the beginner will first meet with upon his first purchase of an instrument, it is the ordinary object glass made for many years, in which a distance always exists between the front lens and the cover glass of the object, or the object itself, this interspace being occupied by air.

Dry lenses are the easier to use, especially the lower powers, as in the majority of cases, the object has only to be placed upon the stage and the objective focussed to it. "Immersion" systems need more care and preparation, and as a rule, are only required to supplement observations first made with a dry lens; they are used by connecting the front lens of the objective to the cover glass of the object, by means of an immersion fluid, so that no film of air exists between the front lens and the object, as in a dry lens. It will be as well to treat of these two classes of objectives separately.

Dry Objectives.—Amongst the dry objectives, the lower powers are often made to separate. An objective giving the amplification of a 2-inch when complete, is converted into a 4-inch by unscrewing the anterior combination. Separating 2-inch and 1-inch, 1-inch and $\frac{1}{2}$-inch, $\frac{1}{2}$-inch and $\frac{1}{4}$-inch are also made, with others; but these separating lenses are not to be greatly recommended.

Tolles, Zentmayer, Wray, Zeiss, Swift, and others, have produced low power objectives, in which the two combinations composing them are separated, or brought nearer to each

other by means of a screw collar, the lens being nominally a 4-inch, a 2-inch, or any intermediate power at will. These lenses define fairly well, and moreover, possess a flat field, so that they may be used for a variety of work where low powers are required.

Low powers are constructed in several ways, according to the aperture desired. Those of greater amplification than the 1-inch, are made either as triplets, or of two pairs of lenses placed at certain distances apart, as all the corrections required are easily made on this system. The triplet, used only for low angles, is the least to be commended, and should not be used with deep eye-pieces. The 1-inch objective may be a triplet, as shown in Fig. 62, or a double combination, as in Fig. 63.

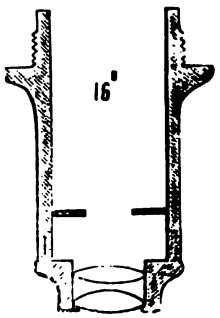


FIG. 62.

In the latter the front is a plano-convex of crown, with a meniscus of flint, being separated by a considerable interval from the posterior combination, which is composed of two double convex crown lenses, holding between them a double concave of flint.

It will be seen from Figs. 62 and 63 that there is more work in the construction of the higher angle. The cost is consequently greater; but when we remember that the 1-inch of 25° admits more light than the one of 16°, that it defines better, resolves better, and proves to be a much superior working glass in every respect, the extra money will not perhaps be grudged for it.

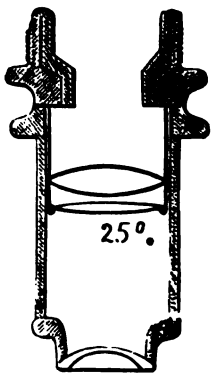


FIG. 63.

Half-inch objectives are made on two systems: the low angles for binocular use, of a thick, solid front, at the back of which are two pairs of partly corrected lenses, the aberrations being finally corrected by the thickness of the front. The higher angles are constructed of three pairs of lenses, the posterior combination being of considerable diameter. They are made of as high an aperture as 0·66 or 82° air angle, while the $\frac{4}{10}$ths of 100° air angle, or numerical aperture 0·76, is far from uncommon.

No objective with an air angle of more than 40° should be used with the Wenham binocular. Dr. Carpenter pointed out long ago the exaggerated effect of projection produced when pollen-grains of the *Malvaceæ* and other similar objects are examined binocularly with high-angle objectives; perfectly spherical objects, instead of resembling a hemisphere, appearing like the small end of an egg.

Powers yielding amplifications ranging between 50 and 200 diameters may be called medium, and are represented in our list by the $\frac{3}{4}$-inch, $\frac{2}{3}$-inch, $\frac{1}{2}$-inch, $\frac{4}{10}$-inch, and $\frac{1}{4}$-inch objectives. The $\frac{1}{2}$-inch is a very handy glass, though it does not seem to be an easy one to construct, judging from many the author has seen. Makers of cheap but really good 1-inch and $\frac{1}{4}$-inch powers seem to fail sometimes in producing cheap and really good $\frac{1}{2}$-inch objectives.

A good working $\frac{1}{2}$-inch may have an aperture of 60°, though it is made of 35° and 40° for special use with the binocular.

Spencer, the celebrated American optician, produces a 1-inch of 50°, but for ordinary work such an objective would not be a desirable investment, as its working distance is extremely short.

It is not to be supposed that all objectives are constructed exactly as shown in Figs. 62 and 63. We have a 1-inch of 34° by the late Robert Tolles, built up of eight lenses combined

in two separate systems, and a four-tenths of ten lenses. The foregoing have simply been given to afford the beginner some idea of the construction of microscopic objectives.

Some of the medium powers are made with conical fronts. Tolles in 1870 seems to have been the first to produce them, having made one in that year for a Mr. King, of Salem, to allow of illumination of opaque objects, with the least obliquity of the illuminating ray to the general surface, and with no injury to the performance of the objective when transmitted light was used. These coned-front lenses are not merely lenses of the usual form in a tapering brass mount, but the front lens itself is a cone, protected in some cases with a thin sheath of metal, made also to project a trifle beyond the glass work to protect it from injury, and in a few cases the front surface was ground slightly concave, for the same purpose.

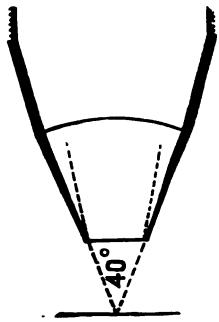


FIG. 64.

The illustration Fig. 64 represents the single front (twice the actual size) of one of Tolles' 40° ½-inch objectives. The angle of the exterior pencil of rays is 40°, the interior pencil 26°, and the focus 0·20-inch distant. The thin sheath of metal is shown turned to a cone of 40°.

FIG. 65.

Fig. 65 exhibits one of Mr. Swift's short-mounted ¼-inch objectives with coned front.

The ¼-inch objective, yielding a power of 200 diameters with the A eye-piece, is a most useful glass to the student, when possessing an air angle of about 100°. It is generally constructed of a triple back lens, a double middle, and a single front. Messrs. Ross's system is believed to differ from this, inas-

much as their objectives, from the ½-inch upwards, are supposed to be constructed on Mr. Wenham's formula, in which the flint concave of a triple middle is made to correct the aberrations of the anterior and posterior crown lenses. An enlarged section of this formula is shown in Fig. 66.

Quarter-inch objectives may be obtained of as high an air angle as 140°, very good for the experienced microscopist, but of limited use to the student, as such glasses focus very close to the object.

All objectives possessing a numerical aperture of more than 0·42 (50°) should be furnished with a screw collar to adjust the lenses for varying thicknesses of covering glass. It has been already mentioned that the late Mr. Thos. Ross discovered that varying thicknesses of covering glass disturbed the corrections of the objective. He also succeeded in devising a plan to overcome this by arranging the front lens so that it might be moved to or from the posterior systems. In most cheap objectives, the front lens (or lenses) is now made to advance from the posterior system, which remains a fixture. This plan is not a good one for high powers, as owing to the extremely small working distance, the front lens is apt to become damaged without very great care be taken in the manipulation, so that all good objectives have the correction collar so fixed that the front lens remains a fixture while the back systems are made to recede.

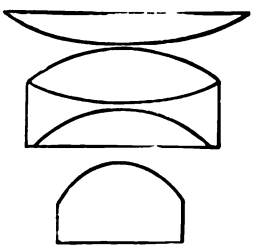


FIG. 66.

Fig. 67 is an illustration showing the details of construction of one of Zeiss' best objectives. The tube C C carrying the two posterior systems is moved to and fro by means of the screw collar E E, the front lenses being fixed into the nose-piece B B; the coiled spring F presses upwards

against the diaphragm and downwards against the tube carrying the posterior lenses. The use of the correction collar will be explained in the chapter on Testing Objectives.

Our remarks relating to the length of the brass-work of objective mounts do not apply to objectives with correction collar; it will be seen from the illustration that a very great portion of the length is taken up with the correction apparatus itself, for which a sufficiently long bearing must be secured.

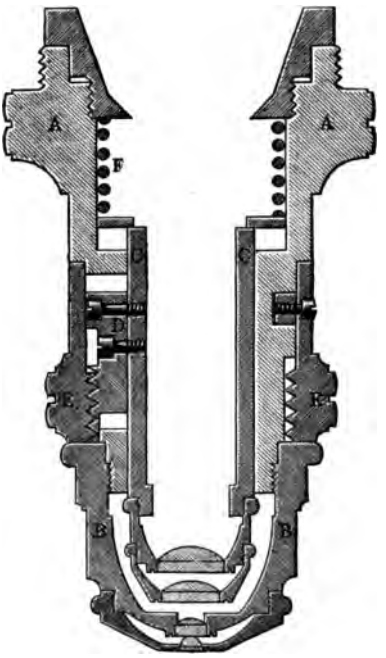


FIG. 67.

Under the denomination of high powers may be classed all those objectives yielding greater amplification with the 2-inch ocular than 200 diameters. They have become very common during the past few years, and very fair one-eighth's for a student's general work, of an aperture of 0·82 (110° air angle), may be purchased for 50*s*.

Speaking as a general rule, it will be found that as the aperture of a dry lens increases, the working distance (*i.e.* the distance between the front lens and the object) will be lessened, though this generalization only applies to those objectives scientifically constructed with some special aperture in view; and taking the apertures into consideration there is not much difference in the productions of our best makers (so far as regards working distance) either at home or abroad.

Upon this point the author speaks advisedly, as when conducting the Verification Department of the "Microscopical News," over four hundred objectives passed through his hands, and an evening was always spent with each, besides the time occupied in making the measurements. We cannot do better than give the following table showing the average measurements of all the dry objectives which were sent in for verification:—

Sold as		At Ten Inches.	e	Real Aperture.	
a Inch.	b Air-angle or Aperture.	d Working Distance. Inches.	$\frac{n\,l}{(n+1)^2}$	f Numerical.	g Air-angle.
	°				°
3	12	1·84	2·15	·075	9
2	16	1·32	1·46	·130	15
1½	14	1·31	1·30	·120	14
1	16	0·75	0·84	·150	17
1	30	0·42	0·80	·240	28
¾	30	0·41	0·68	·260	30
⅔	30	0·33	0·55	·220	26
½	60	0·08	0·36	·540	66
½	40	0·16	0·47	·330	39
$\frac{4}{10}$	60	0·10	0·32	·450	55
$\frac{4}{10}$	100	0·04	0·32	·760	98
¼	70	0·04	0·22	·560	68
¼	100	0·02	0·20	·760	99
⅛	120	0·01	0·14	·820	110
⅛	110	0·016	0·13	·800	106
⅛	140	0·01	0·105	·900	128
$\frac{1}{10}$	135	0·016	0·105	·870	120

The fourth column is the actual equivalent focal length of the objective, as given by Cross' formula; how it is obtained will be explained in the chapter treating of Microscopical Measurements.

Immersion objectives.—Microscopic objectives constructed upon the dry system can never be made to receive rays of greater obliquity than infinitely near, but below, 90° from the axis of the instrument, on account of the great refraction which takes place on the passage of the illuminating rays from glass to air. Very early in the history of the compound microscope it was recognised that wide apertures exhibited more detail in objects, than low angles, and opticians have always striven to approach as nearly as possible the limit they had set themselves of 180° measured in air. Hartnack, a good many years ago, succeeded in producing objectives measuring 170° in air, which were used by placing a drop of water between the front lens of the objective and the thin glass covering the object. We have had several of these early objectives through our hands, but have never met with one possessing a greater aperture than the equivalent of 180° in air, which leads us to suppose that the true principle of the immersion system was not grasped in those days; and even later still, we know it was argued that rays of greater obliquity than 98° in water could not be made to enter an object glass, and further still, that even if such rays could be made to enter, they would be of no use in the formation of an image. Subsequent researches have proved this to be erroneous.

The earliest authentic record we have that the 180° corner had been turned, seems to be in the pages of the "Monthly Microscopical Journal," for March, 1874, where Mr. Wenham gave a description of Tolles' immersion one-sixth objective, for which Tolles claimed an aperture of 98° *in balsam*, and with a working distance of 0·013 inch. The memorable

correspondence which took place concerning this lens led, no doubt, to the solution of the immersion mystery, and furnished many with ideas which have led to important improvements in objective construction.

There is no doubt that the first application of the system of water immersion, by Hartnack, was to secure a long working distance with objectives of wide aperture. As we have already pointed out, as the angle increases, the working distance decreases, so that a dry objective of 170° is an almost unworkable lens. The denser the medium intervening between the front lens and the thin covering glass the greater will be the working distance for the same aperture. Tolles had already noticed this, and though he had nearly reached the goal, by measuring the balsam angle, yet the practical carrying out of the homogeneous immersion system was left for Mr. J. W. Stephenson, and to Professor Abbe of Jena, who was aided in the actual work by Carl Zeiss, the optician. These objectives, calculated by Professor Abbe, had a balsam angle of 113°, and were used by connecting the front lens with the object slide by means of a drop of cedar-wood oil. Now a dry objective with an air angle of 112° (0·83 N.A) will possess a working distance varying from 10 to 15 thousandths of an inch; a water immersion of 112° measured in water (1·11 N.A) will have about the same working distance, while a homogeneous immersion of a similar angle, measured in balsam (1·26 N.A) will vary from 10 to 12 thousandths of an inch. If the numerical aperture is kept constant, then the working distance increases as we go from the lighter to the denser immersion medium, and it is a question whether this property ought not to be more used in the construction of the higher power student's objectives. The Bausch and Lomb Optical Company, in America, furnish water immersions in their "Professional" series giving apertures as nearly as possible to 1·0, and this principle might also be adopted by other makers.

Messrs. Powell and Lealand have recently constructed a homogeneous-immersion objective of $\frac{1}{12}$-inch equivalent focus, with a numerical aperture of 1·43 or 140° balsam angle, with two extra fronts, one of which gives an aperture of 1·28 or 115° balsam-angle, while the other provides an aperture of 1·0, or 82° in balsam. With an aperture of 1·43 the working distance is 0·007 inch; the aperture of 1·28 gives a focal distance of 0·016 inch; while with the numerical aperture of 1·0 the working distance is 0·024 inch.

The following are the measurements of immersion objectives which have passed through the author's hands:—

Sold as		At Ten Inches.	e	Real Aperture.	
a Inch.	b Angle.	d Working Distance. Inches.	$\frac{n\,l}{(n+1)2}$	f Numerical Aperture.	g Angle.
$\frac{1}{8}$	...	·015	·108	·88	124° air angle
$\frac{1}{8}$	112° G	·004	·102	1·20	129 water ,,
$\frac{1}{8}$	...	·042	·091	·76	98 air ,,
$\frac{1}{8}$	1·10	·014	·108	1·12	114 water ,,
$\frac{1}{12}$	116° B	·015	·089	1·16	99 balsam angle
$\frac{1}{12}$	116° B	·015	·082	1·22	107 ,, ,,
$\frac{1}{12}$	112° B	·008	·089	1·26	112 ,, ,,
$\frac{1}{12}$	...	·011	·082	1·25	110 ,, ,,
$\frac{1}{12}$	1·43	·005	·077	1·36	127 ,, ,,
$\frac{1}{18}$	112° G	·005	·061	1·26	112 ,, ,,
$\frac{1}{18}$	112° G	·008	·047	1·26	112 ,, ,,

The first and third lenses in the table were the productions of two of our opticians, made as soon as a demand had arisen for the "aquatic nozzle."

Having become somewhat confused in our nomenclature of apertures, on account of the unequal value of the same as expressed in degrees, Professor Abbe introduced a system based on his own experiments and used in harmony with existing but older optical laws. Under his hand the air angle of 180° was identical with the water angle of 97° and the

balsam angle of 82°, but instead of giving them three separate values he introduced the term "Numerical Aperture," equivalent to 1·0 in each of the three above instances. The *numerical aperture* is easily obtained by multiplying the sine of the semi-angle by the refractive index of the fluid in which that angle has been measured: this is the meaning of the formula:

$$n \sin u = a,$$

where n = the refractive index of the medium; $\sin u$ = the sine of the semi-angle, while a = the numerical aperture.

A table of natural sines for each degree from 1° to 90° may be found in a subsequent Chapter.

The following table has been calculated by Mr. Stephenson, and is reproduced by permission from the Journal of the Royal Microscopical Society:—

Numerical Aperture. ($n \sin u = a$.)	Angles of Aperture ($= 2u$).			Illuminating Power. (a^2.)	Theoretical Resolving Power, in Lines to an Inch. ($\lambda = 0{\cdot}5269\,\mu$ = line E.)
	Dry Objectives. ($n = 1$.)	Water-Immersion Objectives. ($n = 1{\cdot}33$.)	Homogeneous Immersion Objectives. ($n = 1{\cdot}52$.)		
	° ′	° ′	° ′		
1·52	...	...	180 0	2·31	146,528
1·50	...	...	161 23	2·25	144,600
1·48	...	...	153 39	2·16	142,672
1·46	...	...	147 42	2·13	140,744
1·44	...	...	142 40	2·07	138,816
1·42	...	...	138 12	2·02	136,888
1·40	...	...	134 10	1·96	134,960
1·38	...	...	130 26	1·90	133,032
1·36	...	...	126 57	1·85	131,104
1·34	...	...	123 40	1·80	129,176
1·33	...	180 0	122 6	1·77	128,212
1·32	...	165 56	120 33	1·74	127,248
1·30	...	155 38	117 34	1·69	125,320
1·28	...	148 28	114 44	1·64	123,392
1·26	...	142 39	111 59	1·59	121,464
1·24	...	147 36	109 20	1·54	119,536
1·22	...	133 4	106 45	1·49	117,608
1·20	...	128 55	104 15	1·44	115,680
1·18	...	125 3	101 50	1·39	113,752
1·16	...	121 26	99 29	1·35	111,824

Numerical Aperture. ($n \sin u = a$.)	Angles of Aperture (= 2 u).			Illuminating Power. (a^2)	Theoretical Revolving Power, in Lines to an Inch $\lambda = 0{\cdot}5269\,\mu$ = line E.)
	Dry Objectives. (n = 1.)	Water Immersion Objectives. (n = 1·33.)	Homogenous Immersion Objectives. (n = 1·52.)		
	° ′	° ′	° ′		
1·14	...	118 00	97 11	1·30	109,896
1·12	...	114 44	94 56	1·25	107,968
1·10	...	111 36	92 43	1·21	106,040
1·08	...	108 36	90 33	1·17	104,112
1·06	...	105 42	88 26	1·12	102,184
1·04	...	102 53	86 21	1·08	100,256
1·02	...	100 10	84 18	1·04	98,328
1·0	180 0	97 31	82 17	1·00	96,400
0·98	157 2	94 56	80 17	·96	94,472
0·96	147 29	92 24	78 20	·92	92,544
0·94	140 6	89 56	76 24	·88	90,616
0·92	133 51	87 32	74 30	·85	88,688
0·90	128 19	85 10	72 36	·81	86,760
0·88	123 17	82 51	70 44	·77	84,832
0·86	118 38	80 34	68 54	·74	82,904
0·84	114 17	78 20	67 6	·71	80,976
0·82	110 10	76 8	65 18	·67	79,048
0·80	106 16	73 58	63 31	·64	77,120
0·78	102 31	71 49	61 45	·61	75,192
0·76	98 56	69 42	60 0	·58	73,264
0·74	95 28	67 36	58 16	·55	71,336
0·72	92 6	65 32	56 32	·52	69,408
0·70	88 51	63 31	54 50	·49	67,480
0·68	85 41	61 30	53 9	·46	65,552
0·66	82 36	59 30	51 28	·44	63,624
0·64	79 35	57 31	49 48	·41	61,696
0·62	76 38	55 34	48 9	·38	59,768
0·60	73 44	53 38	46 30	·36	57,840
0·58	70 54	51 42	44 51	·34	55,912
0·56	68 6	49 48	43 14	·31	53,984
0·54	65 22	47 54	41 37	·29	52,056
0·52	62 40	46 2	40 0	·27	50,128
0·50	60 0	44 10	38 24	·25	48,200

A popular exposition of numerical aperture was given some years ago by Mr. William Blackburn, the then President of the Manchester Microscopical Society, and as it subsequently appeared in the "Microscopical News," we are enabled to reproduce it, as no doubt it will be readily understood.

In the diagram, Fig. 68, let A O be a ray of light falling

obliquely upon surface of water at O, and refracted in the direction of C, or nearer to the *axis of incidence* B D than the original direction of the ray; then the sine A B of the *angle of incidence* I will be to the sine C D of the *angle of refraction* R as 1·33 to 1·0, and this proportion will be maintained for every obliquity of the incident ray. The number 1·33 is, therefore, the co-efficient of refraction in water or the *index of refraction* for that medium. In like manner the index of refraction for crown glass is 1·52, for cedar-wood oil 1·51, for Canada balsam 1·53, and for bisulphide of carbon 1·68. Now as every ray of light falling upon the point O, within the angle of incidence I, must, in passing

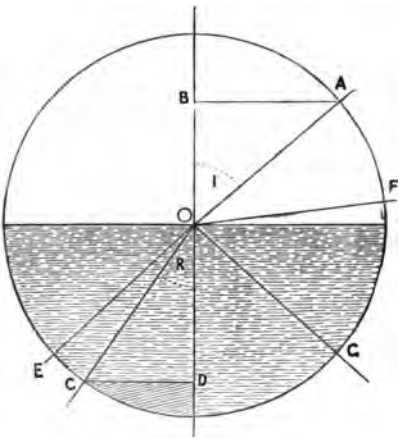


FIG. 68.

into water, be refracted within the angle of refraction R, it follows that the smaller sine C D in water must receive all the light transmitted through the greater sine A B in air; and that equal plane angles in air and water must, under similar conditions, receive amounts of light in the respective proportions of 1·0 and 1·33. Now let the line C D represent one-half of the diameter of the front surface of a water-immersion

objective having its focal point in O, where an object is placed in contact with the immersion medium and illuminated by the semi-angle of light B O A: then, as the amount of light falling upon C D in water is equal to the amount falling upon A B in air, it necessarily follows that the interposition of the immersion fluid between the front lens and the object has increased the quantity of light entering any given semi-diameter of the front surface of the lens in the proportion of the refractive index of water, 1·33; because, if water had not been interposed, the light from the object would have spread over the larger angle E O D, equal to B O A in air; and as the areas of circles are to each other as the squares of their radii, so the amount of light received by the whole surface of the front lens of a water-immersion objective must bear to that received by the surface of a dry objective of similar angular capacity the ratio of the square of 1·33, or 1·77 to 1·0. Similarly in the case of oil as the immersion medium, with an index of refraction = 1·52, the semi-diameter of the front surface of the lens will receive 1·52 times the quantity of light that a similar portion of a lens of the same capacity will receive in air; and the illuminating power of the two lenses will be in the ratio of the square of 1·52, or 2·31 to 1·0. A correct system of notation for apertures will, therefore, require that, when the light passes through different immersion media, the numerical expressions for aperture shall be multiples of the sines of the semi-angles with the refractive indices of those media. This principle is carried out in the system of *numerical aperture*, where *radius*, or the sine of half the angle of 180°, being taken as = 1·0 in air, it becomes = 1·33 in water, and 1·52 in oil.

We have seen that in the diagram, Fig. 68, the ray of light A O, falling upon the water at O, is refracted to C. In like manner a ray of light C O, passing from water into air, will be refracted in the direction O A, the angle of incidence

in this case being R, and the angle of refraction I. Now, if we enlarge the angle of incidence until it is nearly 48½°, we shall find that a ray of light E O will be refracted in such an oblique direction O F, that any increase in the angle of incidence will result in no light passing out of the water, but in the whole of it being totally reflected at O in the direction of O G; and the angle E O D is, therefore, called the *critical angle*, which for water is 48½°, and for crown glass or oil 41°. If the system of notation in *numerical aperture* is correct, therefore, we ought to find that 1·0, = 180° in air, is also equal to twice the critical angles in water and in oil; and we accordingly find that *radius* divided by the refractive index in each case is equal to the sine of the critical angle, and that 180° in air is consequently the same thing as 97° in water and 82° in oil. It will be obvious that any excess over these apertures in water and oil must represent greater apertures than 180° in air.

It may be asked whether we can see these apertures in excess of 180° in air through the microscope; and, if they really exist, what is their practical utility, since dry objectives are made that will take up very wide angles of light, and we have the means of condensing it upon the object by suitable lenses placed beneath the stage. If we take a very wide-angled homogeneous immersion objective (say 1·43 N A) *i.e.*, one constructed upon a formula which requires the immersion medium to be of the same refractive index as the crown glass of which the front lens is composed, and measure its aperture *in air* by means of Abbe's apertometer, we shall find it to be represented by 1·0, or the greatest air-angle of 180°. If we now place a drop of water in front of the objective, we shall find that the aperture has suddenly expanded to 1·33, the numerical equivalent of 180° in water, and one-third greater than the aperture in air. If again, we remove the drop of water and substitute a drop of oil, we shall find that the

aperture has again extended itself, that the full capacity of the objective is now seen to be in excess of 1·33, and therefore greater than 180° in water.

The truth revealed by the apertometer is confirmed in another way. The researches of Professor Abbe, on what is known as the "law of aplanatic convergence," have shown that the aperture of an objective requires a certain ratio to be observed between its focal length and the utilised diameter of its back lens measured at the plane of emergence. This ratio, in a dry objective of 180° air-angle, supposing that angle to be possible, will be as 1 to 2; or, in other words, the focal length will be equal to the semi-diameter, which latter may, therefore, be expressed in terms of the focal length by 1·0. In a water-immersion objective of 180° water-angle, this ratio must be increased by one-third, and the expression for the semi-diameter will be 1·33. Similarly an oil-immersion objective of the same angle will require this ratio to be expressed by 1·52. As these numbers represent also the refractive indices of immersion media in each case, as well as the numerical apertures of the objectives employed, it is very evident that *aperture*, in the sense of *opening* merely, must be compounded of the *sine of the semi-angle* of aperture with the *refractive index* of the immersion medium, and that angles greater than 180° in air or water are not mere theoretical expressions.

CHAPTER VI.

ACCESSORIES.

HAVING made the reader familiar with the compound microscope, its eye-pieces and objectives, we must now consider those appliances which conduce to excellence in microscopical examinations, or which render the work more easy and more quickly performed. It must not be imagined, however, that the possession of all the following accessories is imperative for microscopical studies; far from it, as precisely the same effect as that produced by the most costly accessory can often be obtained (with more trouble) by very simple and inexpensive means.

In the first place, we may consider the draw-tube. It is supplied of considerable length to most monocular microscopes, being cut down in many binoculars.

Into this draw-tube the eye-pieces should be fitted, and the former then becomes a means of increasing the amplifying power of the combination. By simply drawing out the tube the object is thrown out of focus, and the objective has to be carried nearer the object. This wonderfully alters the relative distances of eye-piece and objective from the object (conjugate foci), and the result is a considerable increase in amplification. It must not be forgotten, however, that a limit will soon be found to this method of increasing the magnifying power.

The longer the tube, the more of the periphery of the field will be cut off; and further, the aberrations, which have been fairly corrected for a moderate or short tube, may no longer obtain correction when the body of the microscope is unduly lengthened. Some of these tubes are plain, while others are divided into inches and parts, so that the results once obtained can be recorded if necessary.

There are many uses for the draw-tube, which the student will be sure to find out for himself, and he will find that if it is graduated, as shown in the figure, his work will often be rendered much more easy. Some of its uses will be found described under the subjects of Dissecting and Micrometry.

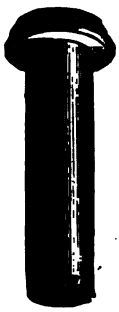


Fig. 69.

Into the lower end of the draw-tube the erector (Fig 69) is made to screw. It consists of a tube about three inches long, having a meniscus (concavo-convex) at one end and a plano-convex at the other, a diaphragm being placed about midway between them. The convex side of each lens is turned towards the eye-piece, this combination producing a second inversion of the image, so that it is seen in its natural position. This is of great use to the tyro dissector, as he has then but little difficulty in the use of his dissecting instruments; but if anyone who has learned to dissect without it should attempt to use the erector, he will find it is quite as hard to unlearn as to learn.

The erector, when screwed into the lower end of the draw-tube, enables the observer to employ a greater range of magnifying power without changing the objective, and this is especially useful in either dissecting or photography by means of the microscope.

The field of view is also much increased, so that a very large object can be included when using the half-inch

objective, and by pulling out the draw-tube (containing the erector, of course) a very considerable increase in amplification is obtained; thus, in the author's dissecting microscope, when the erector is placed as near to the objective as possible, the magnification obtained scarcely exceeds 8 diameters, while when the draw-tube is pulled fully out the amplification is increased to 130 diameters. It should be understood that the defining power of a microscope is not *increased* by the addition of an erector.

The double nose-piece next claims our attention, and was devised by Mr. Brooke for the purpose of quickly changing the objectives without the trouble of unscrewing and screwing up again each time, although it is said to have been used by Professor Bigelow in America a long time before Mr. Brooke. This accessory is made in several forms, but that

FIG. 70.

FIG. 72.

shown in Fig 70 is specially recommended, the straight pattern being sometimes inconvenient, owing to the one objective touching the stage before the other required for use is in focus. It is a very useful addition to the microscope when much work has to be done; but on no account should the beginner purchase one of those made to hold three or four objectives—they are too heavy, and often nullify completely the action of the fine adjustment.

Since 1881 there have been several devices, designed to supersede the double nose-piece, for quickly changing objectives; the chief of them are:—Nachet's, Verick's, Nelson's, and Curties'; but the one we prefer is the "Facility" nose-piece devised by J. L. Pease, of Chicopee, Mass., U.S.A., in 1882, and sold by Messrs. Queen & Co., of Philadelphia.

This appliance has been devised to facilitate the rapid interchange of objectives. The adapter nose-piece A (Fig 71) screws on to the nose-piece of the microscope by the usual "Society" screw, where it may remain permanently. It is

FIG. 71.

provided with mechanism similar to that applied in the "self-centering" chuck. By the partial rotation of the milled collar, three sections of a flat spiral are made to act upon three sprung steel teeth, causing them to project from slots within the cylinder, or to return to their normal positions at will. B is a small ring with which each objective must be provided; it screws on the objective, where it may remain, and on its outer edge is a flanged groove. The objective having the ring B attached, can then be slid into the "Facility" nose-piece, when about one-tenth of a turn of the milled collar on the latter causes the teeth to grip in the flanged groove B, thus securing the objective in place; the

reverse movement releases the teeth from the flanged groove, when the objective will drop into the hand.

Some years ago Mr. Swift introduced a centering nose-piece, which is shown in Fig. 72. In this accessory the objective can be accurately centred after it has been screwed into the nose-piece, which latter screws into the lower end of

Fig. 73.

the body of the microscope. The centering screws are similar to and act in the same manner as those connected with the usual substage. Centering nose-pieces are not very common; the author has seen but one in use, and that with high powers only.

The student will find the bull's-eye condenser (Fig. 78), or stand condenser, as it is sometimes called, a necessary adjunct, an instrument which may be used in a variety of ways; but its proper use, and the methods of illumination generally, can only be completely understood by consulting a few diagrams of mirrors and lenses which show their action upon various rays of light. An illustration of the bull's-eye condenser is given at Fig. 73, from which it may be understood that it is really a plano-convex lens of crown glass, but mounted in various ways, and the student should remember, on purchasing one, to select such a form as may be turned and twisted in every desirable direction.

There are many uses for the bull's-eye condenser. It is an indispensable instrument for the illumination of opaque objects; with it the microscopist is enabled to throw parallel rays on to his mirror from the lamp; he may use it as a spot lens by fixing a small disc of dead-black paper on the flat surface of the lens, and as an Amici prism for the resolution of diatoms, Nobert's test lines, &c.

A smaller condenser, generally called a "condensing lens," or "stage condenser," is supplied with some microscopes, but its use is very limited, and the student is advised to purchase only the independent stand condenser. With the higher powers a more intense illumination can be obtained by a combination of two condensers, the correct method of using which may be made out by inspection of the diagrams in the next chapter.

As to quantity of light, we have always found that students have too much of it, and therefore it may be as well to consider the diaphragm next. The diaphragm is a thin plate of metal perforated with holes of various shapes and sizes, and is used in order to cut off the superfluous rays from an object, or leading to an object, under examination. At one time they used to be placed at considerable distances

beneath the object, but the practice now-a-days is to put them immediately beneath the slide, which doubtless is their proper place.

The ordinary form is shown at Fig. 74, and is called a wheel of diaphragms; it is usually supplied to all student's and third-class instruments.

It is made of many degrees of excellence, and therefore

FIG. 74.

every cheap instrument purchased should be specially examined with a low power (3 or 4-inch objective), to see whether the apertures are concentric with the axis of the instrument. Every part of the diaphragm should be well

FIG. 75.

FIG. 76.

blackened, in order to prevent the presence of reflected light, which would interfere with the illumination.

Collin's graduating diaphragm, illustrated by Fig. 75, was the first of its kind. It consists of several movable shutters, acted upon by a lever, so that the whole of them may be moved inwards or outwards simultaneously. The opening thus produced is not strictly circular, but this is of little importance in actual working.

The graduating or "iris" diaphragm of Messrs. Beck is illustrated in Fig. 76. It differs from that of Mr. Collins only in the number of the shutters, which thereby produce a nearly circular aperture. Mr. George Wale, of New York, supplies a new form of iris diaphragm to all his "working" microscopes.

It is now universally appreciated that the diaphragm must be placed immediately beneath the object, in order to get the best results. This has been attained by the "calotte" diaphragms now made by Messrs. Zeiss, Swift, and others. This form is placed *over* the achromatic condenser, on a level with the stage, as shown in Fig. 77; but the simplest way to effect this is by using a thin plate of aluminium 3-inch by 1-inch,

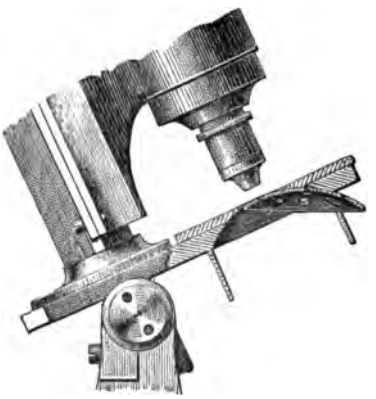


FIG. 77.

with the desired aperture pierced in its centre, and furnished with two spring clips, so that the slide may be placed upon it in any required position, such as has been used by the author for many years past.

There is another method of excluding the excess of extraneous light, used by the author since 1873 with very good results—viz. that of slipping over the objective a small perforated cardboard and aluminium nozzle, blackened inside,

the central hole in the front being just a trifle less than suffices to admit the rays passing from the field of vision.

For many purposes it is necessary to concentrate the light to a greater extent than can be done with a mirror alone, and in such instances an achromatic condenser is usually employed. This may be improvised from an ordinary objective made to screw into an adapter below the stage, a $\frac{1}{2}$-inch or $\frac{4}{10}$-inch objective of wide angle forming a very useful condenser.

A substage condenser consisting of a plano-convex lens of $\frac{3}{4}$-inch focal length was used by Wollaston for illuminating the object upon the stage; this was improved by Goring, but still did not satisfy the requirements of microscopists. In

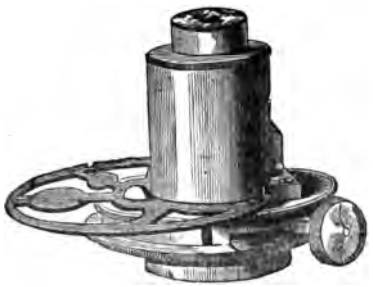


FIG. 78.

1840, the achromatic condenser was introduced, and for many years afterwards continued to be made as a low-angle objective, adapted in various ways to the stage or substage. Whether achromatic light for illuminating purposes is absolutely necessary is a question we cannot discuss here.

Mr. Collins's "Webster" condenser is shown in Fig. 78, and is a form extremely well liked by all practical microscopists; it is made so that the diaphragm may be carried quite close to the lens, and this can be further improved, when dark ground illumination is required, by placing a circular piece of black paper on the lens itself. It can be used with ease in combination with the polariscope.

The use of the condenser may be demonstrated in various ways, but when employed in connection with the diaphragm, one of its uses may be shown by taking a slide of *P. angulatum*, and using the condenser with a small central aperture. In this way it will be found very difficult indeed to resolve the markings properly, but if the central light be stopped out, so as to utilise the oblique rays only, the same effect will be produced as by swinging the mirror round to the side and getting obliquity in that way.

In using the achromatic condenser, great care must be taken to insure the coincidence of the optic axis with that of the microscope, by manipulation of the two adjusting screws

FIG. 79.

of the substage fitting. This is most easily accomplished by the use of the centering glass, made and sold by most opticians.

The complete condenser of Messrs. Swift and Son is a very ingenious piece of apparatus; it is made to supply the place of a substage with all the ordinary compound substage apparatus. It comprises an achromatic condenser with an

aperture of 140°, a spot lens, a contracting diaphragm, a revolving diaphragm and set of stops, polarising prism, together with revolving mica and selenite films. The polariscope is mounted on an eccentric arm, so that it can be thrown in and out of use instantly, which is an important feature, for it may be presumed that the polariscope would be much oftener employed if it could be put in and out of action as easily as shown in Fig. 79.

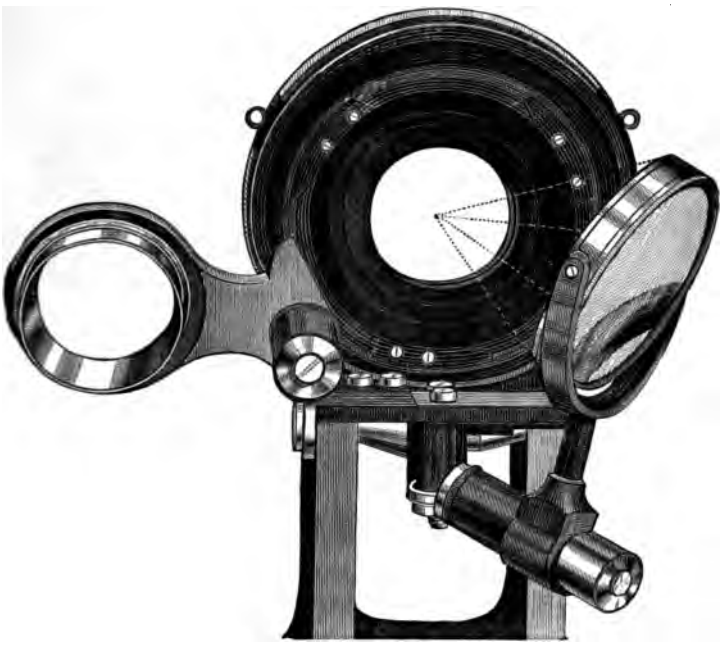


FIG. 80.

In the microscope itself Mr. Swift now inserts the analysing prism of the polariscope in a brass box over the Wenham prism, thus facilitating its use, as there is no unscrewing of parts to fix the analyser in position.

This is also provided for in Mr. Crouch's new method of

adapting the accessory carrier to the under side of the stage, as shown in Fig. 80, from which it will be seen that the condenser, or polariscope, can be quite as easily turned away without dismounting, as in the instruments of Carl Zeiss.

Reade's double hemispherical condenser consists of a plano-convex lens about 1½ inches in diameter, its flat side being placed next to the object, and surmounted by a smaller lens of the same form (hemispherical). A very oblique illumination is possible with this condenser, and as made by Messrs. Ross and Co., it is shown in Fig. 81.

FIG. 81.

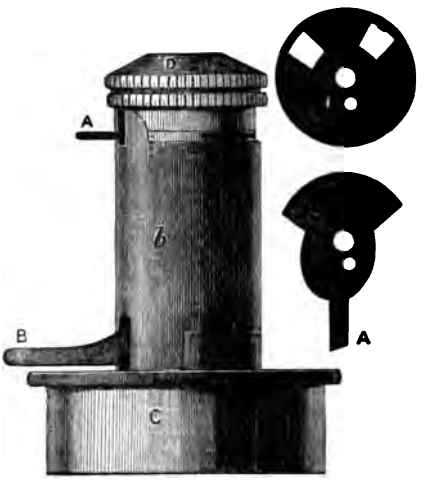


FIG. 82.

Several makers are now producing oil-immersion condensers, in which the under side of the slide is connected with the condensing lens by means of a film of castor oil, oil of cedar wood, or glycerine. That made by Messrs. Powell and Lealand is shown in Fig. 82. It is not achromatic, but is able to utilise rays of 130° balsam angle. Beneath the lens are fitted diaphragms of peculiar pattern, and by its use

the most difficult test objects may be resolved with the mirror in the optic axis of the microscope.

Messrs. Watson and Sons of Holborn produce an oil-immersion condenser, in which the lens is mounted on a plate of ebonite; the object slide being placed above it, the latter is held in position by a pair of clips, and is then ready for examination.

The Bausch and Lomb Optical Company furnish a very useful form of condenser, shown in Figs. 83 and 84.

The lenses composing this condenser are of such a size that they will utilize almost all the rays which may pass through the substage ring. Its numerical aperture is about 1·42, so that it will do justice to objectives of the largest

FIG. 83.

FIG. 84.

angular aperture. Its volume of light is sufficient with the highest amplification, and although it gives an intense light at the focal point, it may be distributed over a larger space by varying its distance from the object. It will work both dry and immersion.

It is supplied in two styles of mountings, with which the same effects may be gained as with almost all the various substage illuminating apparatus. They are light and easily manipulated. Fig. 83 shows the mounting for oblique light; the opening for the passage of light is $\frac{1}{4}$ inch, but may be decreased. It is made to pass slowly from the centre to edge of the mounting by revolving the outside milled edge, thus

giving all angles of light from central illumination to extreme possible limit.

Fig. 84 shows the mounting with a swinging diaphra ring into which may be placed various stops with blue gl which are furnished, without disturbing the position of condenser, and by which dark ground illumination, decrea or oblique light may be gained.

Messrs. R. and J. Beck have recently devised the form condenser shown in Fig. 85, the movable series of front len being mounted in a segment of a sphere and rotated b milled head and pinion acting on a toothed disc. The f lens when brought over the back combination is intended use without fluids. By revolving the diaphragm the angle be varied from 35° to 7°. T next is a full aperture lens, w which, by revolving the d phragm, the angle can be var from 180° downwards. The th lens, with full opening of d phragm, has an aperture of 1· or an angle of 110° in glass; it truncated, stopping off the cen rays. The fourth lens has also aperture of 1·25, and is trunca to stop off all rays up to 180° in air. The fifth is similar No. 3, but the periphery is painted over so as to allow pen only at right angles to pass.

Fig. 85.

One of the most useful forms of condenser is that made Carl Zeiss of Jena, called "Abbe's condenser," the continer pattern of which is shown in Fig. 86, the drawing being a size section of the same.

The upper lens S is a thick plano-convex, somew larger than a hemisphere. Just below it, is a large dou convex lens T, which serves as a collecting lens for S.

illuminating portion is fixed to the upright bar of the condenser, while the diaphragm carrier B is made to rotate upon the pin Z by means of the milled head at *r*. Another illuminating system is shown at Fig. 87, which is made to replace the two lenses shown in Fig. 86.

This condenser has an aperture of 1·4, but when used with objectives of more than 1·0 N.A. (*i.e.*, more than is

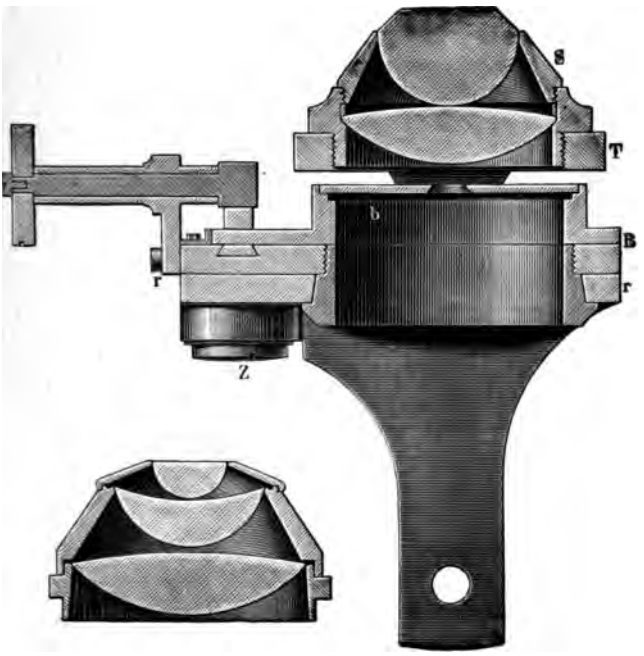


FIG. 87. FIG. 86.

equivalent to 180° in air) the base of the slide must be in immersion contact with the front lens of the condenser. For dark-ground illumination a central stop is placed in B, and Zeiss supplies special diaphragms to be applied at the back of several of his objectives of large aperture, ensuring a dark ground when used with this condenser. Zeiss also makes a

form of the Abbe condenser to fit into the substage ring of the ordinary English stands. This form is shown in Fig. 88, but it is made in such a heavy mounting that it has not found much favour in this country.

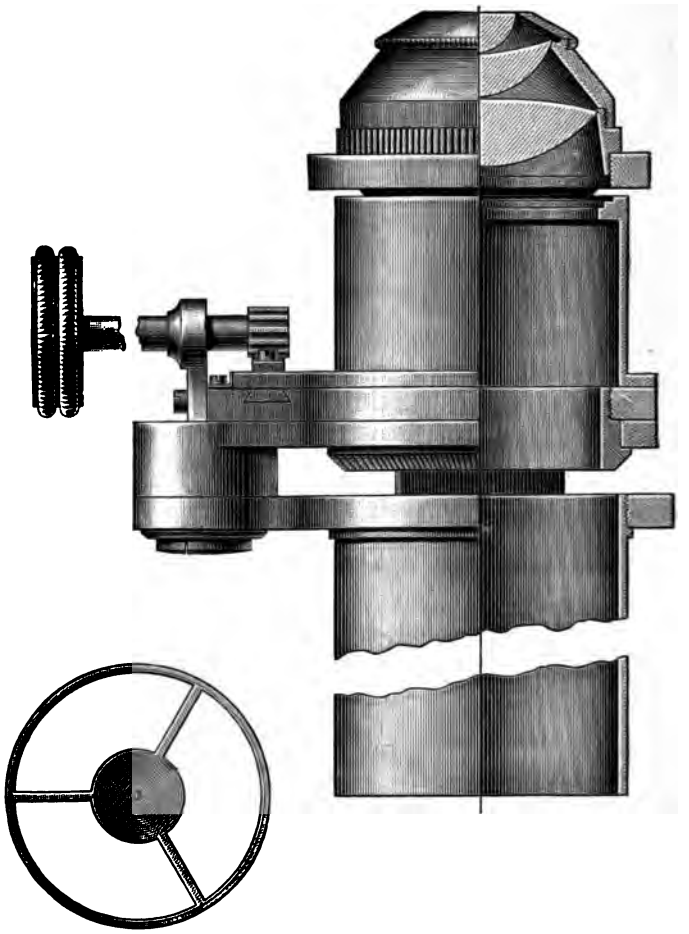


Fig. 88.

The swinging substages of Messrs. Ross and Beck are useful for obtaining very oblique rays of light with the help

of the condenser. This has been accomplished in another way by Messrs. Swift and Son, in their radial traversing substage illuminator, shown in Fig. 89.

This apparatus has been constructed for the purpose of increasing the resolving property of high-power objectives by causing still more oblique pencils to impinge upon the object than can be obtained by most methods. The arrange-

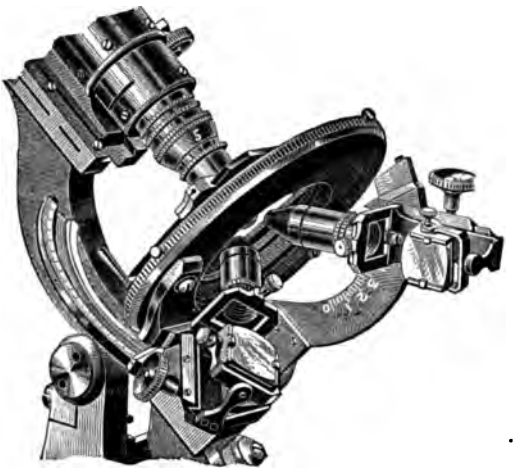


FIG. 89.

ment consists first of an arc-piece, fixed below the stage, radial to an imaginary line drawn through the axis of the microscope objective, in the same plane with the object. On this an achromatic condenser of special construction is made to travel, thus keeping the rays of light on the object during its entire traversing. These rays converge and terminate in a focus through the front lens, in a highly concentrated form. The condenser is illuminated by a rectangular prism, for condensing light into the achromatic combination. The next part consists of a second arc-piece, placed at right angles to

the former one; this also carries a similar achromatic condenser and illuminating prism, which move radial to the centre. Both these arc-pieces are so divided that each pencil of light can be projected at a similar angle, and previous results can always be recorded in the same way. Difficult test objects are readily resolved, especially such diatoms as have rectangular striæ or markings. With a ¼-inch objective, the diatom *Navicula rhomboides* is easily resolved into squares. The markings on *P. angulatum*, by the same means, are made to stand out in bold relief like half spheres. Those usually

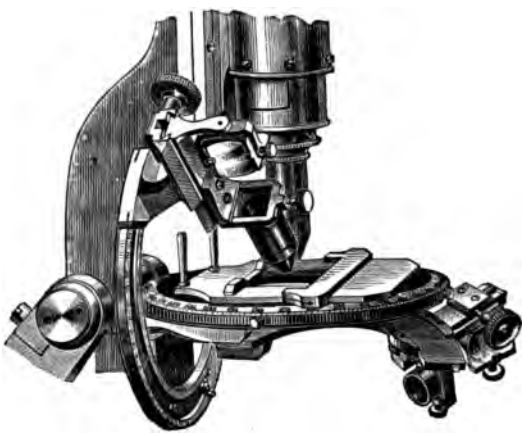


Fig. 90.

considered easily resolvable only require one pencil of light to show the markings. When this is the case, the rectangular arc-piece, with all its illuminating apparatus, can be turned away from the microscope stage, as shown in Fig. 90. The same illustration shows how opaque objects may be illuminated, viz., by moving the condenser of the first arc-piece above the stage of the microscope, when a pencil of light can be projected on to the object more perpendicularly than with the bull's-eye condenser, thus preventing shadows in

coarse or deep objects, which often produce distortion and false appearances. When the apparatus is used for opaque objects, with a lower power than the 1-inch objective, the achromatic combination can be removed and the light directed from the prism, which can be made to give convergent rays sufficient for use with a 4-inch objective.

Wenham's reflex illuminator is valuable for use with high powers; but it possesses the disadvantage that all objects for use with it must either be selected or specially mounted. It has been described by the designer at some length in the seventh volume of the "Monthly Microscopical Journal," p. 237, in which he states that by its use the markings on *A. pellucida* were brought out by an eighth objective, which had never shown them before. Fig. 91 gives a sectional diagram of the illuminator, which has been manipulated from Mr. Wenham's sketch. In using this accessory, the glass cylinder is brought up level with the stage, the centre of rotation is set truly by a dot on the fitting, as seen with a low power. A drop of water is now placed on the top of the cylinder, upon which the slide is laid. With dry objectives, of very wide aperture, the reflex illuminator yields beautiful resolutions of balsam-mounted objects, the field itself being brilliantly lighted up. The plane mirror, or direct light from a low lamp on the table, yields the finest effect upon any object, and by simple rotation of the illuminator, an exquisite unfolding of structure takes place. Objects *on the slide* are brilliantly illuminated, while those on the cover are nearly

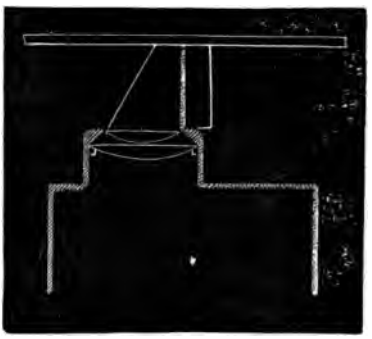


FIG. 91.

invisible. Wenham's reflex illuminator can also be very effectively used in the resolution of test objects by transmitted light, with immersion objectives of wide aperture.

Another illuminator has been devised by Mr. Wenham, and is shown in Fig. 92. It looks very much like the half of a broken button, which, nevertheless, collects and concentrates a surprising amount of light. It consists of a semi-circular disc of glass $\frac{1}{4}$ inch in radius, the edge being well rounded and polished to a transverse radius of $\frac{1}{10}$ inch. This may be obtained from Messrs. Ross and Co.

Fig. 92.

We have heard from several microscopists that they have been unable to use this illuminator successfully, and it has generally been the case where it has been mounted for use in the substage. The best plan is to purchase the article *unmounted*, and to make it adhere, for use, to the under side of the slide, with a little glycerine and gum. This illuminator does not require the addition of any condensing lens, the necessary obliquity of light being readily obtained by swinging the mirror to the side. Mr. Wenham, in writing to the "English Mechanic," says that while he was never successful in the patient manipulation required to bring out the striæ of *Amphipleura pellucida*, yet with the new illuminator he succeeded at once, and on every subsequent occasion.

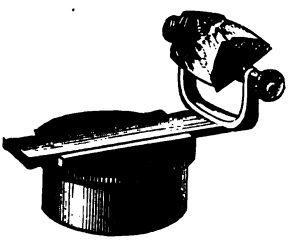


Fig. 93.

The Amici prism ranks next. It is made to exhibit one plane and two lenticular surfaces, thus concentrating and reflecting the rays at the same time. It is mounted so that it may be used in the substage, as shown in Fig. 93, or

better, perhaps, upon a separate stand, so that it is entirely independent of the microscope or stage, Fig. 94.

In using this prism it is set beneath the stage, at such a distance from the axis of the microscope as to furnish rays of sufficient obliquity; and in order to produce the best effect, the stage should be capable of rotation round the fixed luminous rays, as with test objects, such as the silicious valves of diatoms, the *Navicula rhomboides*, for instance, there is nearly always one position in which the markings are shown to the best advantage.

Fig. 94.

Colonel Woodward's prism has been described upon p. 246, vol. I., of the "Journal of the Royal Microscopical Society." It consists of a right-angle prism of crown glass, the long side of which measures $\frac{3}{4}$ inch by $\frac{1}{2}$ inch wide. It is cemented into a base-piece of brass, and supported on a stout steel rod three or four inches long. Colonel Woodward tells us that the whole apparatus ought not to cost more than three or four shillings, but we are afraid he has not consulted the optician in this matter. To use it, the steel rod is slipped into a dark-well holder, and putting a drop of oil on the upper face of the prism, it is placed in contact with the under surface of the slide. The light of a paraffin lamp is then to be condensed upon the object

through one of the faces of the prism, until it is seen, by inspection through the other face, that the objective is well illuminated.

Nachet's prism is shown in Fig. 95, the upper and lower surfaces of which are convex, by reason of which the rays of light proceeding from the mirror are conveyed by the lower surface, and, after undergoing two reflections, are finally brought to a focus upon the object. The prism should be so mounted that it may be revolved for the same reasons as have been already given when treating of the Amici prism. The Nachet prism is not much used in this country, and in its present form it is constructed with the lower surface plane, and not convex as in the original. Then, again, there is the right-angle prism which finds occasional use. The proportion of light reflected by a prism is greater than can be obtained from the ordinary plane silvered mirror, which always yields secondary reflections from the surfaces of the glass.

FIG. 95.

Lord Osborne's illuminator, or diatom exhibitor, is made by Messrs. Baker, of Holborn, and is much praised by many microscopists, as an aid in resolving markings on plane surfaces; it is somewhat similar to Reade's condenser in the form of the lenses, but they are mounted differently.

The subject of condensers and prisms is one which should be well studied by the beginner before he decides to purchase. Always talk the matter over well with experts before committing yourself to any particular form.

The illumination of opaque objects under high powers has been attempted by many, but the most successful appliances for this purpose are easily enumerated—Powell and Lealand's and Beck's vertical illuminators, and Tolles' interior illuminator. The best objects to show the method

of using these accessories the student will find to be the Podura scale and the diatom *Navicula rhomboides*, or the *Amphipleura pellucida*, which will be illustrated in the next chapter.

Messrs. Powell and Lealand's vertical illuminator consists of a highly polished plate of glass, set at an angle of 45° in an adapter, which is screwed on over the objective. There is an aperture in the side furnished with a revolving diaphragm, through which the illuminating rays are made to pass. These rays are reflected downwards, through the objective, upon the object, as may be more easily understood on reference to Fig 96.

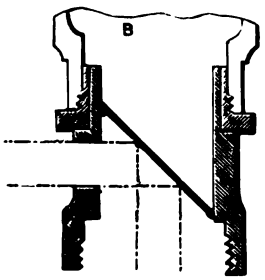


FIG. 96.

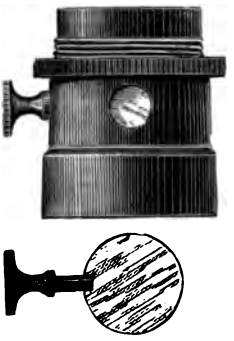


FIG. 97.

Beck's vertical illuminator is very similar to the above, but the plate of glass is replaced by a thin glass disc or thin cover attached to a milled head, as shown in Fig. 97. This seems to be a more handy form than the first, and for ordinary instruments is perhaps the best. Still the author must admit that, with Messrs. Powell and Lealand's workmanship, their form of vertical illuminator is all that can be desired. In December, 1880, Mr. Powell exhibited, at a "Scientific Evening" of the Royal Microscopical Society, *Amphipleura pellucida* lighted up with this illuminator,

and under their new twelfth objective the result was superb.

In order to get the best effect with the vertical illuminator, objectives of very wide aperture must be used. It fails entirely to resolve test objects when they are mounted in balsam, and it is a *sine quâ non* that such objects should be mounted dry and *in contact with the cover.*

The illuminator made by Tolles, and called the "interior illuminator," may be seen in enlarged section at Fig. 98. A prism (A) is inserted between the systems of the objective, which receives and throws down the light through the objective on to the object below. The curves of the lenses figured form that celebrated $\frac{1}{6}$ upon which there was so much controversy as to the real aperture some few years since.

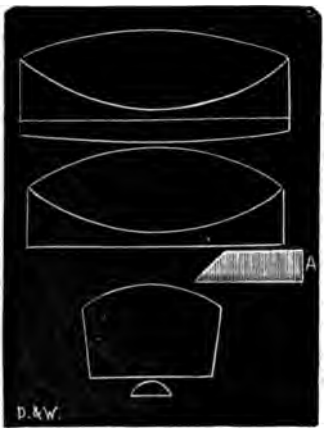


FIG. 98.

Another simple method for illumination under high powers was described by Mr. James Smith, at the March meeting of the Royal Microscopical Society, 1880, extremely well suitable to such objects as Podura scales, diatoms, &c., under powers so high as the $\frac{1}{16}$.

Mr. Smith uses the bull's-eye condenser only, and places it with the plane side uppermost, just above the stage, the lamp being set in front at a distance of two or three inches. The light enters the condenser, and is reflected very obliquely upon the slide from the plane surface of the bull's eye.

An appliance often used to effect dark-ground illumination is the spot lens: it is of very simple construction, being a

plano-convex lens, upon the upper flat surface of which is drawn a circular spot of black varnish, so as to exclude all central rays and prevent them passing into the objective. It is shown in Fig. 99. Messrs. J. W. Queen & Co. make a very useful form of spot lens mounting, so that the lens may be used in instruments of various patterns.

Fig. 99.

Fig. 100.

In the best instruments the paraboloid is the dark-ground illuminator, as rays of greater obliquity can be obtained by the use of this accessory than by the spot lens. It is shown in Fig. 100.

In using the paraboloid, the plane or flat mirror must be used to throw parallel rays upwards, and these are reflected from the internal surfaces of the glass at such an angle that with objectives of moderate aperture the field appears quite dark, the rays simply illuminating the object but not entering the object glass. The track of these rays may be seen on reference to Fig. 101.

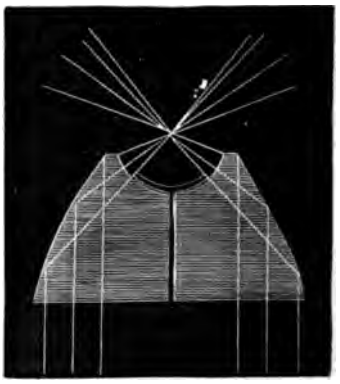


Fig. 101.

As the blackness of the field and intense luminosity of the object depend upon the excess of the degrees of light from the parabola beyond the

angle of aperture of the object glass, as this becomes larger, more of the inner annulus of rays from the paraboloid has to be stopped off, till, at last, with high-angled objectives, it is scarcely possible to obtain a black field, and though this paraboloid may be used equally well for objects in balsam or mounted dry, the utility for its application scarcely extends to object glasses higher than $\frac{1}{5}$, when these are not of large aperture.

A central stop or dark well is placed in the hollow of the paraboloid, and is capable of motion, either upwards or downwards, by means of a pin which runs through a hole drilled in the glass; when this is pushed upwards it cuts off the less divergent rays which would otherwise proceed from the apparatus. When lamplight is used, the bull's-eye condenser should be interposed between the lamp and the plane mirror, in order to parallelise the rays which fall upon the latter, thus yielding really splendid shows with many living objects, such as the Polyzoa; but the *best* results are sometimes obtained by the use of the concave mirror. Practically, when using high powers in conjunction with the paraboloid, to resolve test objects by transmitted light, the *concave* mirror will be found more effective and more easily manipulated. For dark-ground illumination with the 1½-inch to ¼-inch objectives and the paraboloid, most observers invariably use the concave mirror.

When objectives of wide angle are used, a somewhat different arrangement is necessary to secure dark-ground illumination. In the "Monthly Microscopical Journal" for July, 1869, Mr. Wenham reproduces the illuminator which he first described in 1856 as "A method of illuminating opaque objects under the highest powers of the Microscope." Fig. 102 will illustrate the subject of these remarks. Cemented to a glass slide with Canada-balsam is a nearly hemispherical lens "with a segment removed so as to leave

the thickness equal to about one-third the diameter of the sphere. The circle formed by the removal of the segment is blackened in order to exclude all rays below the incident angle of total reflection. This lens is intended to be held in conjunction with the paraboloid, as shown in Fig. 102. The rays pass through the lens in a radial direction without refraction, and proceed until they reach the upper surface of the thin glass cover, where they are totally reflected upon the object."

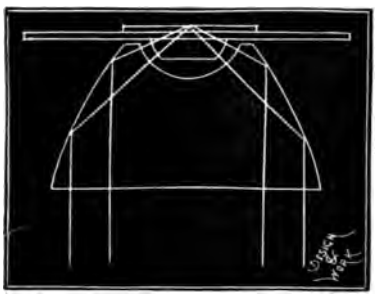


FIG. 102.

The paraboloid, immersion paraboloid, and Wenham's reflex illuminator can be very effectively used in the resolution of test objects by transmitted light, in conjunction with objectives of the widest aperture.

An immersion paraboloid has since been devised by Dr. Edmunds, and made by Messrs. Powell and Lealand; it is somewhat similar to the above in principle.

For the illumination of opaque objects several kinds of apparatus are used, and of these we may consider first, the parabolic reflector as made by several opticians. This is most useful for low powers, from the 2-inch to the $\frac{2}{3}$, and is made to fasten on to the objectives by means of a spring clip, so that it may slide up or down, or be turned round to secure the best illumination.

When this reflector is used with a lamp, the bull's-eye condenser must be placed between them, with the flat surface towards the lamp, and its focal point so adjusted that the rays emitted from the bull's-eye shall be parallel. The chief use of this reflector is to reflect side light from all points of its surface to a point situated on the plane of the base in the focus of the objective. The form as made by Messrs. Beck is illustrated in Fig. 103.

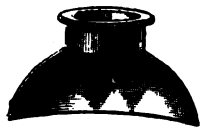


FIG. 103.

At A, B, C, and D, Fig. 104, are shown the accessories called Lieberkuhns, or Lieberkuhn's reflectors, which are used occasionally for the vertical illumination of opaque objects. They are small silvered concave mirrors mounted

A. B. C. D.

FIG. 104.

on short tubes, to admit of adjustment on the objective, and thereby yielding the maximum illumination. A separate Lieberkuhn is required for each object-glass, as the focus of the concave mirror has to be adjusted to that of the objective.

In using this appliance, parallel rays of light are thrown through the stage by means of the plane mirror, which, on meeting the Lieberkuhn, are deflected upon the object, as shown in Fig. 105. Lieberkuhns are not much used at the present time, and can only be applied to objects which are so mounted as to allow of the passage of marginal rays through the slide.

Side reflectors, which are much used for the illumination of opaque objects, are either mounted in the same manner as

stage-forceps, or, as shown in Fig. 106, in a collar which spans above the objective, as made by Mr. Swift.

The side reflector may be used to illuminate opaque objects on the stage very successfully, and with such obliquity that many delicate markings which are unobserved with the Lieberkuhn are brought out without difficulty. In use, the bull's-eye should be interposed between the reflector and lamp, to render the light parallel, exactly the same as with the parabolic reflector.

FIG. 105.

FIG. 106.

When the object is mounted on an ordinary slide, as a transparent object, a dark ground may be produced by using one of Lister's dark wells, which are merely blackened stops, their deep cup-shape insuring perfect blackness, their diameter

intercepting the rays which would otherwise pass into the objective. The engraving (Fig. 107) is an illustration of a dark well in its substage mounting, as made by Messrs. Beck.

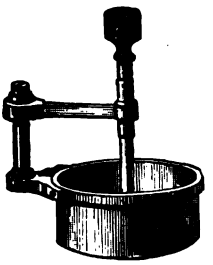


Fig. 107.

For these dark wells the author often uses small circles of black paper mounted on the ordinary 3-inch by 1-inch slips, and for most purposes they answer admirably.

We now come to several very simple but important additions to every microscope, the first of which is generally supplied with all instruments. Fig. 108 delineates the stage forceps, shown holding a fly for rough examination under low powers. They are useful chiefly to beginners, with such objectives as the 3-inch or 2-inch, but are altogether unsuitable for high powers.

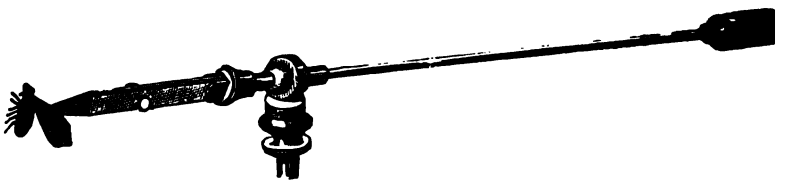


Fig. 108.

In the investigation of minerals it is often necessary to examine small angular pieces which require to be viewed on all sides. In order that this may be done easily, Messrs. Beck make what is called a stage mineral-holder (Fig. 109), one of the jaws being movable in a right line, so that it may clamp any sized specimen, and by turning the milled head of the jaw the mineral is made to revolve. Fig. 109 will perhaps show more clearly the action of this holder than any description can do.

Morris's rotating stage (Fig. 110) often serves the purpose of stage forceps. In its improved form it can be used for

Fig. 109.

both opaque and transparent objects. Small flies, larvæ, beetles, &c., can be affixed to the cork by means of a small pin, or with gum, and as the stage moves upon a secondary plate by means of a ball-and-socket joint, the object can be

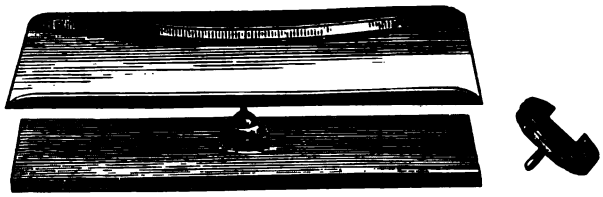


Fig. 110.

placed in a variety of positions hardly possible by any other means except the disc-holder of Messrs. Beck.

Beck's disc-holder, shown at Fig. 111, is for the purpose

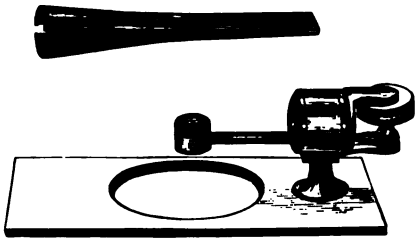


Fig. 111.

of holding for examination under the microscope the small discs upon which objects have been temporarily or per-

manently mounted. The object is attached to the disc by means of gum, or any other suitable adhesive material, and when placed in the holder for observation the disc can be rotated in both vertical and horizontal directions. These discs are very useful for many objects, especially those not needing a cover, and Messrs. Beck supply boxes into which they fit when not in use.

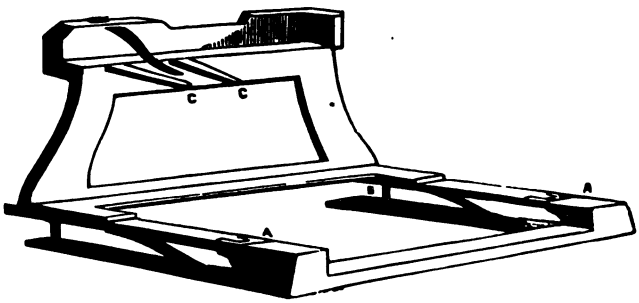


FIG. 112.

In working with high powers and expensive slides there is often a risk of one or the other getting damaged, and this is especially the case with immersion objectives, where the kindly help of the dust on the cover-glass is not obtainable. It is never advisable to take high powers or rare slides to conversazione or other public meetings, on account of the miscellaneous character of observers; but if such a proceeding is imperative, the exhibitor should certainly provide himself with one of Stephenson's safety stages, shown in Figs. 112 and 113, which may be

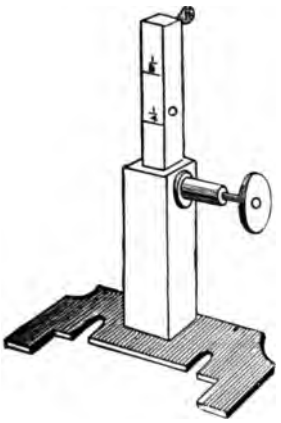


FIG. 113.

the means of saving him a few regrets. It is so constructed that if by chance the object-glass is racked down on the thin cover, no damage is done, on account of the object receding as soon as contact is made, the springs shown in the figure making that motion possible. The object is placed on the two short arms C C, and is held in its place by the spring, which is placed above and between them. In order to make safety doubly sure Mr. Stephenson has devised a second piece of apparatus to act with the former; it is shown in Fig. 113, and consists of a square rod of brass which must be adjusted to suit the various objectives used; it is held in its place by a pin passing through it, attached to a screw at the outer side of the socket in which the rod slides. This instrument is placed (in the Ross model) beneath the bar which carries the microscope body, and, when properly adjusted, allows the objective to touch the object upon the stage, but arrests all further progress, no matter with what degree of force the coarse adjustment may be pressed, a property of considerable value to public exhibitors.

Another very useful accessory is the revolving table, several forms of which are now sold at a very cheap rate. At one time the cheapest which could be obtained was about £9, and now they may be procured (with a slate top) for less than one-fourth of the sum. (Fig. 114.)

When two or more microscopists are pursuing any investigation together, the constant rising from chairs must often have been thought a nuisance, but a good revolving table enables mutual observations to be made with comfort. The author's revolving table is 2 feet 4 inches in diameter, the top of it is 2 feet 3 inches from the ground, and four or even six observers may comfortably sit round it.

And now a few words as to illuminating apparatus. The best light which can be obtained is that from a good white cloud on a sunny day; but, unfortunately, in our towns and

crowded cities we get but little sunlight undiluted with smoke, and students generally are occupied the day through, so that it becomes necessary to use artificial light.

When using light from the sky or from the sun, it should be remembered that the rays are, for all practical purposes, parallel, and thereby differ essentially from artificial light, the rays of which converge strongly from the luminous centre. For use at home there is nothing, perhaps, so convenient as

Fig. 114.

an argand gas reading lamp, sliding up and down on a metal rod, with a shade over it to prevent extraneous light from reaching the eyes. Students are very apt to work with too much light, and thereby impair the sensitiveness of their eyes; they should endeavour, however, to work with only just as much light as is necessary to bring out plainly the details of the object under examination, *and no more.*

If an oil lamp is desired, a very common one may be

made to answer almost every ordinary purpose, provided it is low enough, as when it is required to be raised, that may be readily accomplished by means of blocks of wood of varying thickness. The ordinary form of microscope lamp is shown in Fig. 115; it differs slightly in construction in the hands

FIG. 115.

FIG. 116.

of different makers, but the student should eschew all forms in which the oil reservoir case is *soldered* to the sliding ring; all the author has seen from different makers have come to pieces in a very short time.

A rather better lamp than the above for general work is that of Mr. Swift, and shown in Fig. 116, which, however,

in the writer's estimation, would be much improved by a glazed porcelain chimney, for in most examinations (perhaps all) it is a very important point to avoid a flood of extraneous light passing to the eyes.

The light is more intense when the *edge* of the flame is turned towards the object to be illuminated; but if quantity of light is required rather than intensity, the flat side of the flame may be so disposed.

There are several other lamps which may be mentioned here: Collins's Bockett lamp and Fiddian's lamp, made by Messrs. Ross and Co. The Fiddian lamp is supported by a massive claw stand, from which rises a vertical support on a ball-and-socket joint. A brass tube slides on the vertical rod bearing the condenser and lamp, with neutral tint shade and "white cloud" reflector, having telescope and clamping screw adjustments. When these are placed in any desired relation to each other, the whole can be vertically adjusted by a rack and pinion with the greatest accuracy. Its price is five guineas. Another beautiful lamp has been made by Messrs. Wood, of Liverpool, for Messrs. Dallinger and Drysdale. These two observers, whilst working at the life-history of the monads, appreciated the difficulty of accurately centering the image of the flame when working with the $\frac{1}{25}$ and $\frac{1}{50}$ objectives, and so devised this lamp, which is illustrated in the April number of the "Monthly Microscopical Journal" for 1876, vol. xv.

Parkes' microscope lamp, with cooling evaporator, may be seen in Figs. 117 and 118. C is a bronzed copper cylindrical shade $3\frac{1}{4}$ inches in diameter, with a hood at the front to prevent the upward reflection of light. At the back is a parabolic reflector transmitting nearly parallel rays, made removable for the purpose of cleaning. At the front is a tinted "light modifier," secured by a bayonet joint, and may be also removed when desirable. D (Fig. 118) is the "cooling

evaporator"; a layer of thick felt is placed inside for saturation. When the lamp is lighted this vessel is filled with water, and so prevents the radiation of heat upon the observer's head. The felt requires moistening about once every five hours.

FIG. 117.

FIG. 118.

Mr. Bullock, of Chicago, and Messrs. R. and J. Beck, of London, have lately brought out new forms of lamps which promise to be of great use in pathological and physiological investigation. That made by Messrs. Beck is shown in Fig. 119. The illustration shows clearly the chief features of the lamp without any lengthy description. It may be necessary to say, however, that the chimney F is of thin brass, with two opposite openings closed by ordinary 3 × 1 inch glass slips.

A semi-circle swings from the two uprights G, to which it is attached by the pins H, level with the middle of the flame; to

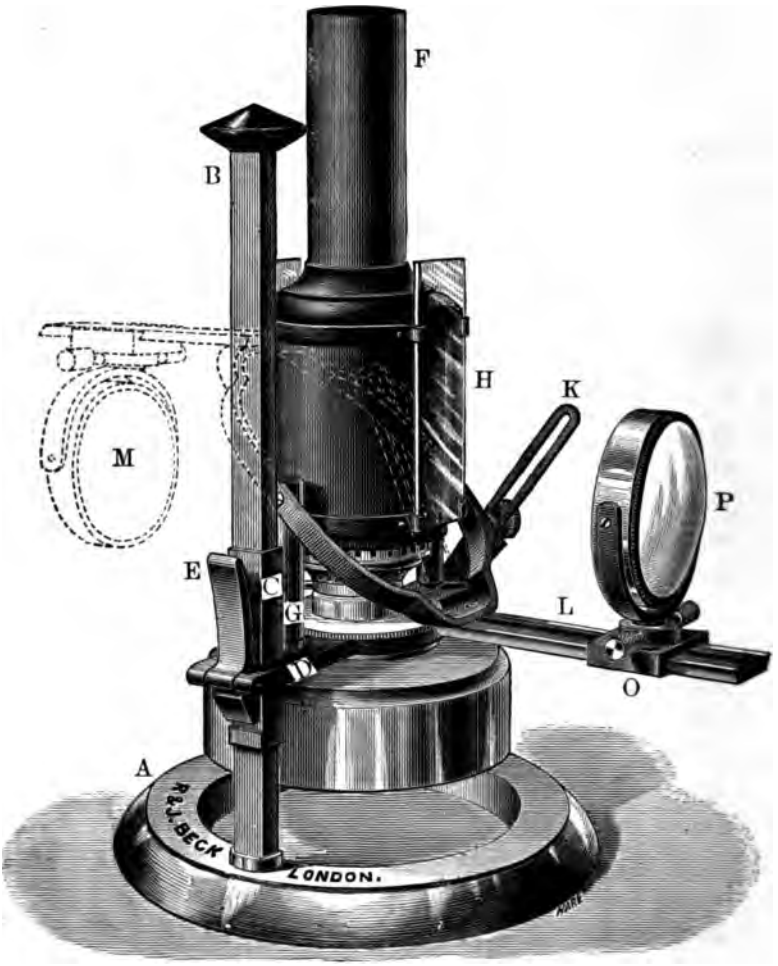


FIG. 119.

this semi-circle is fixed a dovetailed bar L, carrying a sliding fitting O, which bears a condenser at P.

This condenser swinging with the middle of the flame as

a centre is always at the same distance from it, and thus, when once focussed, needs no further alteration for any change in the inclination of the beam of light. This lamp will be found very generally useful.

The light of the sun, a white cloud, or the electric light, which the author has used, each give a light of remarkable purity. This is not the case, however, with the light from gas or from oil lamps. These last, especially gas, give a very objectionable yellow tone, while some tints are nearly suppressed. This effect has been noticed by all observers, and in 1872 Mr. Collins produced a light-corrector, and exhibited the same at a soirée at the Quekett Club. It consists of a brass stage-plate with a groove in which rotates a diaphragm of four apertures—one open, one fitted with a finely-ground glass, while the others are fitted with two different tints of blue. Rainey produced a light-modifier before this, but it was of such construction that it required fitting to each microscope; that of Mr. Collins, on the other hand, can be used with any instrument, and without fitting.

The effect of the blue glass is to effectually correct the yellowness proceeding from all artificial illumination, rendering the light soft and agreeable, and is said to improve the definition as well. To produce this effect the author employs a simple 3-in. × 1-in. slide of blue glass, such as is used by the chemist for the qualitative analysis of potash salts.

Dr. Woodward prefers to use the ammonia sulphate of copper cell, and then only for high power definition, and he says he has been able to resolve the markings on *Amphipleura pellucida* with objectives found incapable of doing it with white light. Professor Smith, of Ashtabula, also expresses his approval of the use of monochromatic light. He says that with its use, and an eighth dry objective, he has easily resolved the *A. pellucida* to beads, in balsam, with deep eye-

pieces; and with the lowest eye-piece the transverse and longitudinal striæ were easily seen.

The white cloud illuminator is a contrivance made in order to produce the same kind of illumination from artificial light as is obtainable from a white cloud. It is generally used with low powers only, and is made in several ways—a concave surface of plaster of Paris, a mirror coated at the back with zinc-white paint, roughened enamel, and white paper have been used to produce this effect, as well as the disc of ground glass found in Mr. Collins's light-modifier.

Thus closes the chapter on accessories; but the student must not think we have exhausted the subject: there are many pieces of apparatus in occasional use which it has not been thought necessary to include here, and many others will be described in the subsequent chapters under the headings with which they are more intimately connected.

CHAPTER VII.

GENERAL REMARKS—TEST OBJECTS.

WHEN we consider the many adjustments of apparatus needed ere a correct picture of an object can be placed before the eye, it will be readily seen how necessary it is to pay strict attention to details—more especially of illumination, this being one of the first and most important lessons the microscopist has to learn.

When rays of light pass through media with parallel faces, such as the glass slips used by every worker with the microscope, the emerging rays are parallel with those entering, the immediate portion being bent away from both these planes, as shown in Fig. 120. If water be used above the glass, the emergent ray will be bent up more towards the perpendicular, while when cedar-wood oil, or any of the homogeneous-immersion fluids are employed, the path of the ray will be one continuous line from the under side of the glass slip. A diagram of the passage of a light-ray through glass is in Fig. 120.

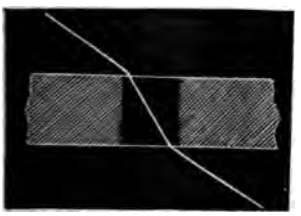


FIG. 120.

Ordinary glass slides, and also the thin covers, are made from crown glass having a refractive index varying from 1·5 to 1·525 referred to air as unity: the following table of mean refractive indices of many substances used by the microscopist may not be uninteresting:—

REFRACTIVE INDICES.

Air	1·000
Water	1·336
Sea water	1·343
Alcohol	1·373
Glacial acetic acid	1·380
Equal parts, glycerine and water ...	1·400
Glycerine	1·475
Oil of turpentine	1·478
Crown glass	1·5 to 1·525
Homogeneous-immersion fluid	1·500
Chloride of cadmium in glycerine ...	1·500
Cedar-wood oil	1·512
Canada balsam	1·532
Flint glass...	1·575
Monobromide of naphthaline	1·658
Bisulphide of carbon	1·678
Oil of anise	1·811
Sulphur	2·115
Phosphorus	2·224

Plane or flat mirrors reflect an image of the same size as the object, the flame of a gas or oil lamp, for instance; the rays are parallel, and the image not inverted. When light is reflected from glass, the under side of which is silvered, much of it is lost, from several causes; but when polished metal is employed for the reflecting surface, the rays do not enter the substance of the reflector, and there is less loss of light than in the former instance.

We must now turn our attention to the concave mirror, with which all respectable microscope stands are furnished. In this kind, the focus is situated at a point at which the reflected rays meet, and when rays parallel to the axis are brought together after reflection, the meeting point, or focus,

is at an equal distance between the centre of curvature, C, and the mirror itself; and, consequently, if a luminous object be placed in this principal focus F, the rays emitted by the whole surface of the mirror will be parallel, as seen in Fig. 121.

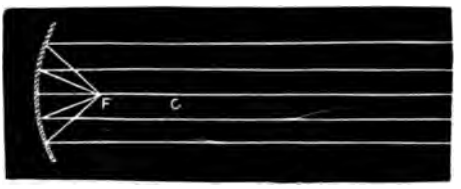


FIG. 121.

If, however, the luminous point be at a greater distance from the mirror than the principal focus, or *vice versâ*, if the luminous rays fall divergent upon the concave mirror, a focus is obtained at another point, called the conjugate focus. In the first case the rays, instead of being parallel, will converge towards L (Fig. 122), while in the second the focal

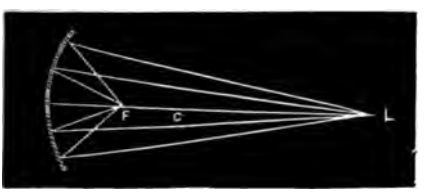


FIG. 122.

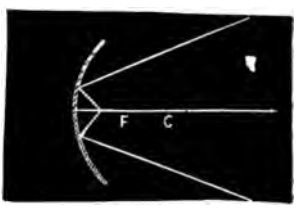


FIG. 123.

point of light will be removed further away from the mirror, and the rays proceeding from a lamp may be brought to a focus until the distance between the source of illumination and the mirror has been lessened to the centre of curvature, the rays being then reflected on to themselves.

If the source of light be placed between the principal focus and the mirror, the reflected rays will be divergent, as shown in Fig. 123.

Let us now consider the action of lenses upon illuminating rays. In a double convex lens the refracted rays from a parallel pencil of light form a focus very near to the centre of curvature of the lens, and conversely when a lamp is placed in its principal focus, a double convex lens may be made to appear the source of light, as shown in Fig. 124.

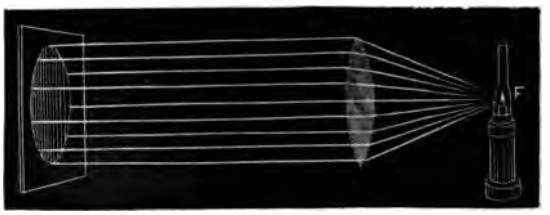


Fig. 124.

It will be seen that a double convex lens has a principal focus on either side of it, and therefore the light may be parallelised on either side; but if the source of illumination be placed further away than the principal focus, the rays will be no longer parallel, but centred in a point at some distance from the opposite side of the lens, as shown in Fig. 125.

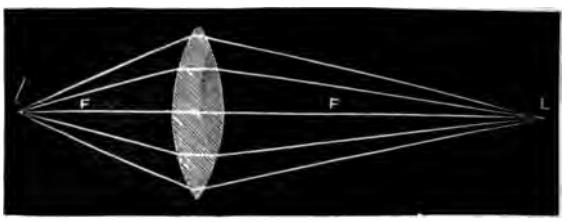


Fig. 125.

These points (*l* and L) are called conjugate foci, and do not lie in any fixed plane, but are dependent the one upon the other; it is this movement of the conjugate foci which yields a long working distance from the objective when the body of the microscope is shortened, and requires the object-

glass to be approached nearer to the object when the draw-tube is used.

Diverging rays can be produced by placing the illuminating point between the principal focus and the lens, and when converging rays fall upon a double-convex lens they are brought to a focus at a point between the principal focus and the lens itself, as shown in Fig. 126.

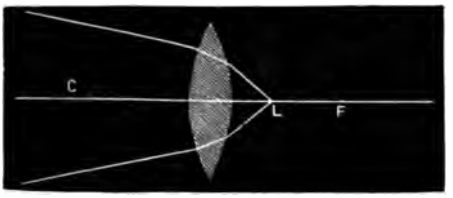


Fig. 126.

The action of a plano-convex lens, of which our bull's-eye condenser, Fig. 73, is a type, may be studied in the same diagrammatic manner. This may be considered as a double-convex lens split down the centre, and so forming two plano-convex lenses; it is generally used for purposes of microscopy on account of the great working distance of its

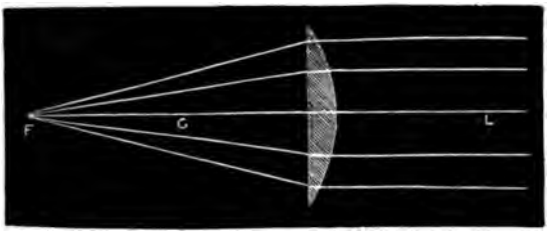


Fig. 127.

focus. As already described, parallel rays falling upon a double-convex lens come to a focus very near the centre (radius) of its curvature; but when the same rays fall upon the curved surface of a bull's-eye condenser, they are brought to a focus at a distance equal to the *diameter* of the curvature,

or twice the distance of a double-convex lens, as may be seen in Fig. 127; and, conversely, if we wish to produce rays of parallel light from a lamp, the ordinary bull's-eye condenser must be placed twice as far from the luminous point as would be necessary in the case of a double-convex lens, a condition of extreme importance when we consider the heat given out during combustion in most oil lamps.

These remarks upon the behaviour of certain lenses and mirrors towards the rays of light may be considered superfluous, nevertheless, as the student proceeds, he will find that not one word too much has been written. False appearances are often produced by the bad employment of light, and the student is advised to practise many kinds of illumination upon objects with which he may be familiar, so as to acquaint himself with the various appearances which diverse applications of it will afford.

When the action of the various mirrors and lenses has been fairly grasped, the student should proceed with some work capable of giving him experience of the manner in which various objects are delineated or depicted under various objectives. In the days of Dr. Goring (1832), when objectives even of high amplification had not surpassed an air angle of 55°, and even when achromatics were despised by nearly all working microscopists, it would not have been a difficult task to test an objective for its spherical and chromatic aberrations, by means of the tests we now possess; but time has changed all things microscopical; really bad lenses are rarities, and taking objectives too, and including the $\frac{1}{4}$-inch, it is remarkable how few imperfections they possess

In order to correct perfectly the aberrations of objectives, the practical optician employs the globule of mercury, or "artificial star," as a test object, while the accuracy of the setting is examined by studying the reflected image of a

flame or the window bars, while the mount with lenses *in situ* is revolving in the lathe.

Beyond mention of it, the "artificial star" test need not be described here: it has been fully treated upon by Dr. Goring in the "Microscopic Cabinet," to which the reader is referred, while the introduction of a good series of test objects renders the general employment of the former scarcely necessary.

It must not be forgotten that great differences exist between test objects. Amongst the slides of *Pleurosigma angulatum*, sold for this purpose, some are so extremely coarse as not to be a test in any sense of the word, while others are so finely marked that they can only be resolved with the greatest difficulty under a $\frac{1}{4}$-inch objective of high air angle. This is also the case with the diatoms *Navicula rhomboides* and *Amphipleura pellucida*, used as tests for the highest powers; so that but little reliance should be placed upon statements that such and such a diatom was resolved under a certain objective.

Object-glasses for use with the microscope are usually spoken of as possessing the following qualities:—

1. Working distance of the front lens from the object;
2. Defining power;
3. Flatness of field and freedom from distortion;
4. Penetrating power;
5. Resolving power;

and it is to ascertain their excellence, or otherwise, and in these directions that test objects are brought into use.

Working Distance.—It has already been shown that the nomenclature of objectives does not presuppose any working distance from the front lens; in fact, such a thing would be impossible, seeing that the enlargement of the aperture reduces the distance of the front lens from the object, the amplification remaining the same. The designa-

tion, such as a "1-inch objective," indicates only that such an object-glass should possess the same magnifying power as a single lens of 1-inch focus, the distance the front of the system focusses from the object not being considered at all.

Great working distance is valuable in an objective only when circumstances demand it. Thus, for dissecting, or for the examination of opaque objects, a certain amount of distance is requisite for manipulation and illumination; but when an object has been prepared and mounted, no more working distance is absolutely required than will admit of the use of the thickest covering-glass, and of the examination of a moderate depth of object.

High-angle objectives of low power and consequent shorter working distance will define much better than the smaller apertures, and there is sufficient working distance, even for dissecting, with the 1-inch of 30° air angle.

The working distance of objectives varies very much with different makers. We have but once seen the nominal power of a lens exceeded in working distance, and that was in a 1½-inch objective, by the late James Smith, of London. The lens measured 1·3 inch by Cross's formula, and possessed a working distance of 1·31 inches. The largest working distance we have met with in a 1-inch objective has been 0·81 inches, the air angle 22°, the maker, Dancer, of Manchester. Tolles' 1-inch of 34°, in the possession of the author, has a working distance of 0·39 inch, while a 1-inch of 28° of Englebert and Hensoldt measured only 0·37 inch working distance.

There is some difficulty in selecting a ¼-inch objective. A glass of 40° air angle possesses considerable working distance, being a power well suited for dissections; while a ¼-inch objective of 80° air angle scarcely gives 0·03 inch of working distance, focussing closer to the object than an ordinary ¼-inch objective of 85°.

Half-inch objectives, with the same apertures, vary quite as much as the lower powers, according to who the maker has been. The largest working distance given to an air angle of 40°, which has come under our notice, has been 0·25 inch, the smallest, 0·12 inch; while the author's ½-inch, by Browning, has an angle of 60°, and a working distance of 0·15 inch.

With the ¼-inch and all higher powers the working distance is very small, so that the microscopist is often precluded from using covering-glasses of the ordinary thickness. This is the case with all extremely high apertures used as dry objectives. Immersion objectives, for the same apertures, afford much longer working distances than dry lenses, so that it is often possible to use an immersion $\frac{1}{16}$-inch where the covering-glass is too thick for a dry ⅛th.

The author would like to see objectives catalogued by the makers in somewhat the following manner. They are actual numbers taken from the verification department of the "Microscopical News"; the oculars, too, might be included:—

Designation.	Aperture.		At ten inches from Micrometer to Screen.		Price.	Remarks.
	Numerical.	Air-angle.	Working Distance. Inches.	Real Amplifying Power.		
1 inch	...	16	0·750	0·861	...	Triplet
,,	0·29	34	0·392	0·864	...	Four system
½ inch	0·35	41	0·250	0·608	...	For binocular
,,	0·50	60	0·150	0·411	...	With correction
,,	0·63	78	0·032	0·302	...	Dry objective
¼ inch	0·42	50	0·062	0·263	...	,, ,,
,,	0·94	140	0·015	0·204	...	,, ,,
$\frac{1}{16}$ inch	1·00	180	0·022	0·089	...	Wat. immersion
,,	1·25	...	0·012	0·082	...	Homog. ,,

DEFINING POWER.—This property is of the first importance in objectives, and has been described by Dr. Goring

to mean "nothing more than a destitution of both kinds of aberration." A well-corrected objective focusses the individual rays of a pencil of light, both from the centre and periphery, to the same plane as shown at F in Fig. 128, but, nevertheless, a more or less distinct image is produced for some distance from each side of this focal plane. If, how-

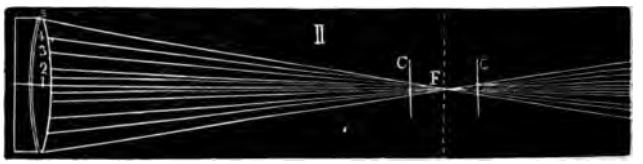


FIG. 128.

ever, the rays are not thus corrected, the outlines of edges of the image will be thick and confused, and the glass is said "to be wanting in definition."

This fault may be shown diagrammatically in Fig. 129, which is an under-corrected glass, the peripheral rays being brought to a focus at *f f f*, between the central focus F and the lens itself. Lenses in their primitive state are very

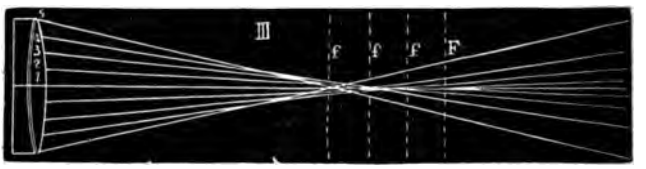


FIG. 129.

much "under-corrected," and can only be employed when it is possible to cut off the peripheral rays by a diaphragm.

An over-corrected lens is shown in Fig. 130, from which it may be seen that the marginal rays are thrown further away from the glass, being brought to a focus at *f f f* respectively, while F represents the focus of the central portion.

An objective free from both these defects is said to be free from spherical aberration—or aplanatic.

These defects in cheap low-angle objectives are corrected (if the term will apply) by the interposition of a diaphragm behind the back lens cutting off the marginal rays. These rays cannot then enter into the formation of the picture, the result being a dark, not very well defining glass of low angle, but long working distance, though not sufficiently corrected to work with deep eye-pieces. In the best objectives of high angle the marginal rays are not cut off, but corrected to the very edges by the application of a wider back lens than usual; the aperture is therefore larger, but the objective possesses less penetration than one of lower aperture, and the working distance has been materially reduced.

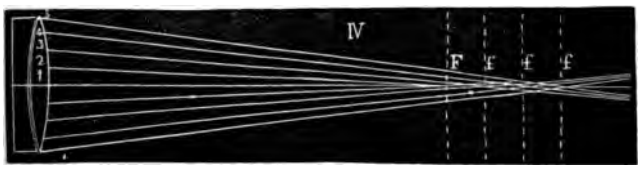


FIG. 130.

A small aperture objective may be constructed from one of these more perfectly corrected lenses by the addition of a diaphragm; such an objective would bear deep eye-pieces and possess a fair amount of penetration, though the working distance remains unaltered, owing to the thickness of the lenses and their various curves not being specially planned with this end in view.

The defining power of an objective may be examined by the employment of certain test objects obtainable from Mr. Cole or Messrs. Watson, until the student has learned how to prepare them for himself. The pollen of the Hollyhock (*Althea rosæ*), shown in Fig. 56, is a useful test; it must be

illuminated as an opaque object, and with deep eye-piece (the Huyghenian D) the minute spines should be readily and clearly defined.

A well-cut wood-section, such as is shown in Fig. 131, is also an excellent test of definition. The borders of each vessel and cell should be clearly and sharply delineated; there must be no mistiness or blackness of edge. A dark image shows at once that too much of the peripheral pencil has been cut off, the definition of a small aperture objective being *never* equal to one of large angle, that is, supposing the workmanship to be equally good in both cases. The figure shows a section of the Horse-chestnut stem (*Æsculus hippocastanum*).

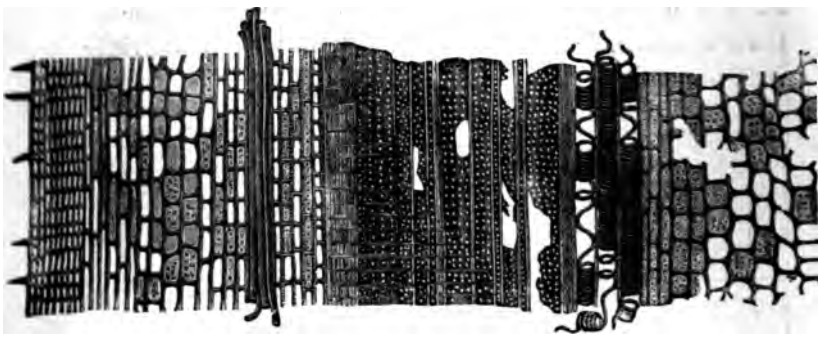


Fig. 131.

Triplets, such as are shown in Fig. 62, generally break down under deep eye-pieces.

The student should, if possible, compare this object under two objectives, one of foreign make of low angle yielding an amplification of 50 diameters with the A eye-piece, the other a 1-inch of English construction, possessing an air angle of 30°.

In order to discover how the correction for colour has been performed, several objects may be employed. Dr. Carpenter's test is the section of pine-wood shown in Fig. 132; it should be mounted dry, and the small circles (*glandulæ*) must be well defined and free from colour even

with the D eye-piece. Perhaps *absolute* freedom from colour under deep eye-pieces does not yield the utmost perfection in resolution; but authorities differ on this point.

For higher powers a white petal of the Pelargonium may serve as a test object, it is a moderately severe test for a ½-inch objective; while for higher powers still, the diatom *Meridion circulare* will show directly whether the corrections have been well executed.

Insect scales are generally used for judging the defining

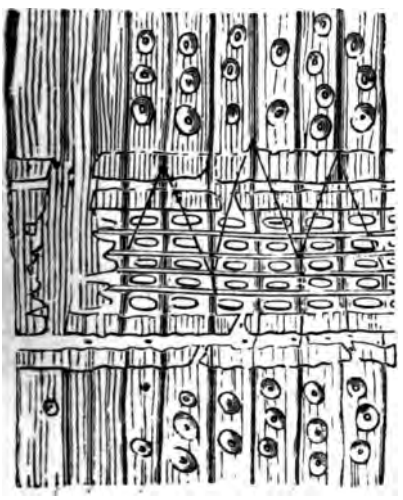


Fig. 13

Fig. 133.

power of higher objectives, such as the scale of the *Morpho Menelaus*, shown in Fig. 133, and that of the Podura, Fig. 134.

The former exhibits lines in a longitudinal direction with transverse markings, attributed to the corrugation of the internal surfaces of the lining membrane, and which are only to be noticed by the use of a good objective.

The Podura scale forms an excellent test of definition; it was known for this purpose before the appearance of Prit-

chard's "Microscopic Cabinet," published in 1832; but the true character of the markings was not then known. This scale was then included amongst the "line tests," whilst now, with objectives of wide aperture, it presents the appearance shown in the figure. Central light from an achromatic condenser is very good for exhibiting this scale, or it may be illuminated as an opaque object by means of the lamp and bull's-eye condenser; but perhaps the best sight of this scale

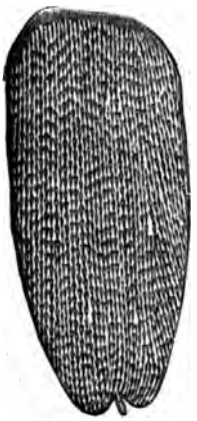


FIG. 134.

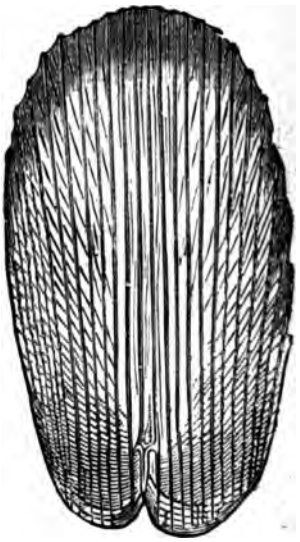


FIG. 135.

is to be obtained by the use of an oil immersion one-twelfth objective, the scale itself being illuminated by means of an immersion paraboloid.

Other insect scales are to be found in use as test-objects, such as those of the *Lepisma saccharina*, shown in Fig. 135, and the battledoor scales of the *Polyommatus argus* delineated by Fig. 136.

These are considered much easier tests than those of the *M. Menelaus* and Podura (*Lepidocyrtus curvicollis*), but not

so variable in quality. Fig. 137 shows one of the ordinary scales of the *Morpho Menelaus*, being, however, magnified to but one-half the extent of Fig. 136.

In the use of these test objects, great attention must be paid to the illumination, and more particularly to the adjustment of the lenses for the thickness of the covering glass. But little correction is needed with the screw collar adjustment in the water-immersion objective, and still less, if any, is required with the homogeneous system; nevertheless, it should always be added, as then correction is possible, should

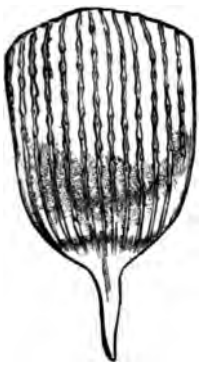


FIG. 136.

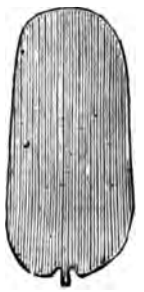


FIG. 137.

it ever be required to meet exceptional cases. Great care should be taken in properly adjusting objectives by means of the collar correction before submitting them to any of the foregoing tests. If the thickness of the covering glass is unknown, the operation will be one of considerable difficulty; but the observer should always have ready prepared a series of test-objects, under covers of known thickness, so that he may be familiar with the appearances produced by a disturbance of the corrections of the objectives. Zeiss marks the correction apparatus in terms of the thickness of the covering

glass, so that with all his lenses it is only sufficient to know this to approximately make the necessary correction.

It must not be forgotten that either lengthening or shortening the body-tube of the microscope, from the normal length for which the objective was corrected, disturbs the

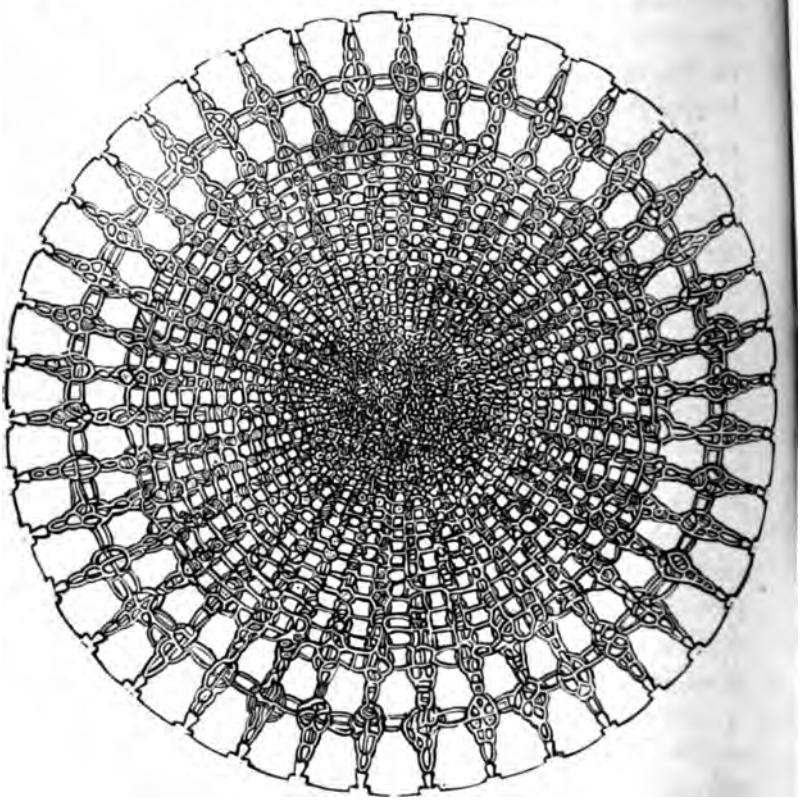


FIG. 138.

balance of the corrections, but this, in a great measure, may be overcome by the proper use of the correction collar.

3. FLATNESS OF FIELD AND FREEDOM FROM DISTORTION.—These properties in objectives may be tested in several ways; for low powers, a section of a large spine of

Echinus, such as shown in Fig. 138, may be used; a well-cut and perfectly flat wood-section; or perhaps better still, one of Mr. Dancer's exquisite micro-photographs. The whole field should be well defined under one focussing, the margin as well as the centre. For higher powers similar objects may be used, but, of course, of smaller dimensions, while freedom from distortion can be tested for, by observation of the micrometer placed upon the stage, or a series of ruled lines varying from one thousand to ten thousand to the inch.

4. PENETRATING POWER.—"Penetration," as it is understood now, signifies that property which an objective possesses whereby several planes of an object are brought into focus simultaneously. Until very recently, the property of *penetration* was shrouded in mystery, and its usefulness often exaggerated; but, thanks to Professor Abbe, who has made it a special study, most of the difficulties surrounding the subject have now been cleared away.

The greatest use of "penetration" is, perhaps, in the employment of the binocular microscope, for it is only when an object can be seen in its entirety that a true stereoscopic image can be obtained. There is no doubt also that this penetrating power is very useful for general work, such as photography or dissecting; still, it should be remembered that a low-angle lens with much penetration will not usually stand deep eye-pieces.

To anyone who has mastered Professor Abbe's elaborate papers on the subject of penetration in objectives an explanation will be unnecessary, but to many it may be an aid, by freeing the matter from the greater portion of its technicalities, and it is hoped will also act as an incentive to the study of "penetration" and "aperture" a little deeper than is usually the case.

In making microscopical measurements the most convenient unit to adopt is the *micra*. This unit is the thousandth

part of a millimetre, or the $\frac{1}{25400}$ part of an English inch; but if a proper appreciation is obtained of the magnitude of the *micra*, it is unnecessary, nay, injurious, to hamper one's mind with the knowledge that it is a fraction of either of the above linear standards.

The student of microscopy should consider the micra on its own merits, as a minute standard of measurement, the magnitude of which may be appreciated by the knowledge that the small diameter of some species of bacteria is equal to one micra, the spores of the mould fungus *Penicillium glaucum* 3·0 micras, the long diameter of tous-les-mois starch about 96 micras, while the diameter of many of the Foraminifera reach from 300 to 800 micras. For brevity the word micra is usually written μ.

Now, Professor Abbe has told us that the penetration of the microscope depends principally upon three factors:—

1. The accommodation of the eye.
2. The focal depth of the lens itself.
3. The medium in which the object is immersed.

So that a practical turn may be given to the subject by manipulating the figures given by Professor Abbe, on page 680, vol. i., series ii., of the "Journal of the Royal Microscopical Society." There is little doubt that such a method as this will help the student to understand the true value of penetration in by far the best manner; and may possibly give a hint to the photomicrographer as to the lenses he must employ when it is desired to photograph solid forms. It will be readily understood that when it is desired to discern pyramidal forms the penetration must necessarily enable a well-defined picture to be taken from apex to base at one focussing; but when the object is of spherical nature the penetration need only approximate to little more than half the diameter, unless it is wishful to show the uppermost layer of the preparation, as well as the slide itself, simultaneously.

I. The "accommodation depth" may vary a little according to the sensitiveness of the observer's eye, and other physiological conditions; but with the same observer the only serious quantity we have to consider is the amplification, and we shall find that a 4-inch lens, magnifying 10 diameters, with the A ocular, yields us a penetrating power from the eye alone of 2,080 micras; a $\frac{1}{10}$ of an inch gives only a penetration of $\frac{1}{5}$ of a micra.

II. The "focal depth" of the objective depends upon the aperture employed for producing the various amplifications. A 4-inch, of 16° air-angle, would have a focal depth of 262 micras, while a $1\frac{1}{2}$ inch objective, of the same air-angle, yielding an amplification of 30 diameters, could only penetrate through 86 micras.

A direct comparison of several objectives of different apertures and varying amplifications, may with advantage be introduced here, and the table goes to show that what is seen down the tube of the microscope results from the combined effects of the eye and the objective, but when a picture is thrown upon a sensitive plate, it is evident that the first quantity is nearly eliminated, and the only penetration attainable is that which the lens itself possesses. The objectives are each and all supposed to be used with the A ocular.

From the following table it will be seen that large objects cannot possibly be penetrated even with objectives of low angle and medium power. The seeds of the *Betula alba* measure 1,100 micras across them, and require, therefore, 550 micras of penetration to see the whole of one of them under one focussing. This amount cannot be obtained from a $1\frac{1}{2}$ inch objective of 16° air-angle, even allowing the 230 micras, which the accommodation of the eye affords, and if we wish to photograph such an object as the above, the 4-inch of 8° air-angle will not be found possessing sufficient focal depth.

Inch.	Air-angle.	Aperture.	Penetrating Power $\frac{1}{a}$	Focal Depth in Micras.	Accommodation Depth of Eye in Micras.	Total Depth of Vision in Air. Micras.
	°					
4	8	·07	14·30	522	2080	2602
4	16	·14	7·19	262	2080	2342
1½	16	·14	7·19	86	230	316
1½	20	·17	5·75	69	230	299
1½	24	·21	4·81	57	230	287
½	40	·34	2·92	10·6	20	30·6
½	70	·57	1·74	6·3	20	26·3
½	110	·82	1·22	4·4	20	24·4
⅛	74	·60	1·66	1·99	2·3	4·29
⅛	100	·76	1·31	1·57	2·3	3·87
⅛	...	1·20	·83	·99	2·3	3·29
$\frac{1}{12}$	110	·83	1·20	·72	·58	1·30
$\frac{1}{12}$	144	·97	1·02	·61	·58	1·19
$\frac{1}{12}$	...	1·10	·91	·54	·58	1·12
$\frac{1}{20}$	160	·98	1·02	·37	·21	·58
$\frac{1}{20}$	...	1·10	·91	·33	·21	·54

The Foraminifer *Orbulina universa*, a beautiful spherical body with fine surface markings, is now before us. It possesses a diameter of 600 micras, and consequently 300 micras of penetration are necessary to see the whole under one focussing. The 1½ objective and A ocular magnifying together 30 diameters will just suit this, provided it does not possess an angle of over 20° in air, but if we wish to photograph this spherical body a much lower power than the 1½ inch must be employed, as the focal depth of this objective is not higher than 86 micras. *Orbulina universa* affords us good proof of the accuracy of Professor Abbe's figures. Under the 1½ inch objective of 16° and the A ocular the spheres are splendidly seen, and the same may be said of the 2 inch of 16° and B ocular; but when the picture is thrown upon a ground glass screen the want of penetration is soon apparent, for it is only when the amplification of the picture has been reduced to rather less than 10 diameters that a satisfactory result is obtained.

Similar illustrations may be offered of the higher power

objectives, the larger species of Polycistina require a depth of 75 micras to show them distinctly, whereas a half-inch objective of 40° in air, when used with the A ocular to produce 100 diameters of amplification, possesses but 10·6 micras.

A one-sixth objective, magnifying 300 diameters, loses exactly one μ in depth between 74° air-angle and 1·2 numerical aperture, so that while the spores of *Penicillium glaucum* (diameter of spores 3 μ) could be photographed with the former, it would be impossible to obtain perfect sharpness with the latter.

The figures in the Table for the $\frac{1}{12}$ and $\frac{1}{20}$ objectives are equally confirmed by the results obtained in practice. The short diameter of *Bacterium termo* may be taken as ·8 μ requiring a penetration of ·4 μ to yield a clear picture, and this is obtainable by using a homogeneous $\frac{1}{12}$ of 1·10 N. A to produce an amplification of 600 diameters. A $\frac{1}{20}$ objective, magnifying 1,000 diameters, although producing a fairly sharp picture to the observer's eye, cannot produce an equally sharp image on a prepared plate, as the focal depth of such an objective will only approximate to ·37 μ, and this statement is borne out by the photographs published by Dr. Sternberg in his translation of Magnin's Treatise on the Bacteria, wherein those pictures taken with a Beck's $\frac{1}{5}$ are much clearer though smaller than the plate taken with Zeiss' $\frac{1}{18}$. There is more detail in the latter, and here comes in the value of amplification and aperture.

There is a third element which goes to make up penetration in the microscope. The depth of vision increases in direct proportion with the refractive index of the mounting medium. Thus, if a combination of lenses possessed a penetrating power of 100 micras when used over an object mounted dry, that depth would be increased to 133 μ when mounted in water, to 147 if mounted in glycerine, to 153 μ in balsam, 168 μ if in iodide of potassium and mercury, and to

222 μ when it is mounted in phosphorus. The great gain in stereoscopic effect, on objects mounted in a medium of high refractive index, has led Mr. E. Ward, of Manchester, to mount opaque objects in balsam, with extremely good results.

It will be seen that the accommodation depth is very great with low amplifications. Under a magnifying power of 10 diameters, it amounts to nearly 2·1 millimetres, while with the higher powers, the image passes quickly into a mere section. The depth of focus does not diminish at such a rapid rate, but the thickness of an object which can be seen under one focussing, decreases accordingly as the amplification increases, and therefore it is most important, where binocular vision is essayed, to use the lowest power sufficient for distinctly recognising the object; and with transmitted light to employ as narrow a pencil as will sufficiently illuminate it.

The following table by Professor Abbe will sufficiently illustrate these remarks:—

Amplification. / 0·50 N.A.	*a*. Diameter of Field.	*b*. Accommodation Depth.	*c*. Focal Depth.	*d*. Depth of Vision. $\frac{b \times c}{b + c}$	Ratio $\frac{a}{d}$ to *d*.
	mm.	mm.	mm.	mm.	
10	25·0	2·08	0·073	2·153	11·6 to 1
30	8·3	0·23	0·024	0·254	32·7 ,,
100	2·5	0·02	0·0073	0·0273	91·6 ,,
300	0·83	0·0023	0·0024	0·0047	176·6 ,,
1000	0·25	0·00021	0·00073	0·00094	266 ,,
3000	0·083	0·00002	0·00024	0·00026	319 ,,

The higher the numerical aperture of an objective, the less will the penetration be, though the defining and resolving power, with quantity of light admitted, increase with the absolute aperture of the objective, provided the aberrations are well corrected.

The following table (see next page) has been abstracted

from the "Journal of the Royal Microscopical Society" for August, 1881, wherein may also be found Professor Abbe's paper, from which we have largely drawn.

Numerical Aperture. ($n \sin u = a$.)	Penetrating Power. $\left(\frac{1}{a}\right)$	Illuminating Power. (a^2)	Numerical Aperture. ($n \sin u = a$.)	Penetrating Power. $\left(\frac{1}{a}\right)$	Illuminating Power. (a^2)
1·52	·658	2·310	1·00	1·000	1·000
1·50	·667	2·250	0·98	1·020	0·960
1·48	·676	2·190	0·96	1·042	0·922
1·46	·685	2·132	0·94	1·064	0·884
1·44	·694	2·074	0·92	1·087	0·846
1·42	·704	2·016	0·90	1·111	0·810
1·40	·714	1·960	0·88	1·136	0·774
1·38	·725	1·904	0·86	1·163	0·740
1·36	·735	1·850	0·84	1·190	0·706
1·34	·746	1·796	0·82	1·220	0·672
1·33	·752	1·770	0·80	1·250	0·640
1·32	·758	1·742	0·78	1·282	0·608
1·30	·769	1·690	0·76	1·316	0·578
1·28	·781	1·638	0·74	1·351	0·548
1·26	·794	1·588	0·72	1·389	0·518
1·24	·806	1·538	0·70	1·429	0·490
1·22	·820	1·488	0·68	1·471	0·462
1·20	·833	1·440	0·66	1·515	0·436
1·18	·847	1·392	0·64	1·562	0·410
1·16	·862	1·346	0·62	1·613	0·384
1·14	·877	1·300	0·60	1·667	0·360
1·12	·893	1·254	0·58	1·724	0·336
1·10	·909	1·210	0·56	1·786	0·314
1·08	·926	1·166	0·54	1·852	0·292
1·06	·943	1·124	0·52	1·923	0·270
1·04	·962	1·082	0·50	2·000	0·250
1·02	·980	1·040			

From the foregoing considerations, "tests" for penetration would seem to be superfluous, seeing that it results from two almost fixed conditions; nevertheless, it is well to know how it may be observed. A section of frog's lung or of human liver, is useful for this purpose, as, when mounted, the various parts will be found to have contracted somewhat, producing corrugations or folds, and consequently the rays proceed from different planes, so that the test should be to see how much or how little of the total depth can be seen under one focussing, without indistinctness.

As an instance of the work specially suited for low angles and consequent penetration, the cyclosis in *Vallisneria spiralis* is often cited. The author can assure his readers that this may be easily seen with advantage under a ½-inch objective, of 80° air angle (0·64 numerical aperture), the largest yet made for this power in this country.

Some twelve years since, when the enlargement of aperture of the objectives was being effected, Mr. J. B. Dancer, of Manchester, applied a graduating diaphragm of square aperture to the back of the objective mount. This, he discovered,

FIG. 139.

gave a certain amount of penetration, and so devised the two different patterns shown in Figs. 139 and 140; and when, some years after, Professor Abbe published the fact that penetration decreased as aperture increased, several of the leading opticians furnished a small aperture diaphragm with wide-angle objectives; but nothing seemed to have been written to propagate the doctrine that penetration could be obtained from wide-angle objectives.

For some years past the author has been in the habit of reducing the aperture by means of perforated metallic discs, and these finally gave way to an iris diaphragm placed above

the objective, as shown by Fig. 141. The construction of this "aperture shutter" is much like the aberrometer of Dr. Piggott, but its application is quite distinct.

Starting, we will presume, with a ½-inch objective, of 75° air angle, by gradually closing the shutter, there will be found a position at which the whole depth of a moderate-

Fig. 140.

sized Foraminifer may be seen at one focussing, and a wide-angle 1-inch may be similarly made to show the whole of such a fungus as *Myxotrichum deflexum*.

Fig. 141.

It appears that Mr. W. H. Bulloch, of Chicago, constructed a very similar shutter to that shown in Fig. 141 as early as 1878, to be used above the objective, but the author has been unable to discover that it was designed with a view to give penetration to wide-angle lenses.

5. Resolving Power.—Without entering into any of the theories of this property in objectives, it may be briefly stated

that it depends entirely upon large aperture, combined, of course, with accuracy of the corrections for sphericity and chromatism. The several pieces of apparatus mentioned in the previous chapter are often essential with objectives of wide aperture, for it is obvious that the advantages would be lost if sufficiently oblique rays did not enter into the formation of the image. The Ross-Zentmayer stand, used with a 1-inch or 2-inch objective as a condenser,

FIG. 142.

FIG. 143.

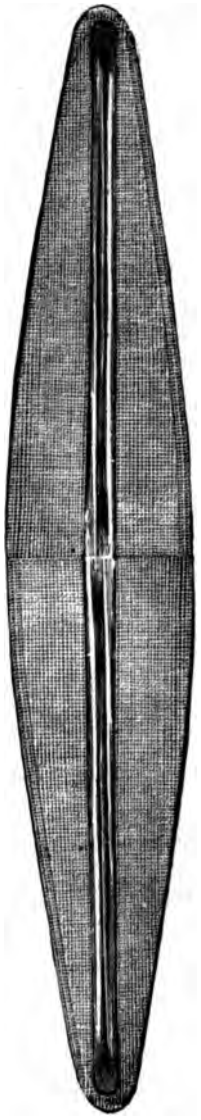


FIG. 144.

FIG. 145.

the radial sub-stage condenser of Messrs. Swift and Son, or the oil-immersion condenser of Messrs. Powell and Lealand, are all capable of producing light-rays of great obliquity.

Diatom frustules, as a rule, furnish tests for the resolving property of medium and high-power objectives, to which may be added the insect scales already shown; but these natural tests are all of very variable quality. *Pleurosigma formosum* and *P. angulatum*, shown by Figs. 142 and 143 respectively, are tests of the resolving property of the ½ and ¼-inch objectives, both of which have been engraved from photographs taken by the late Dr. Redmayne, of Bolton.

Navicula rhomboides, shown in Fig. 144, is used as a test for the ⅛th objective and higher powers. It is rather a difficult diatom to resolve properly without accessories, but with the Powell and Lealand oil-immersion condenser it may be managed without much trouble.

Amphipleura pellucida, shown in Fig. 145, is a most difficult diatom to resolve; indeed, it cannot be accomplished by any dry objective save of the widest aperture, and even then it requires most careful attention to the details of illumination.

Dr. Woodward considers this frustule to be the most useful test for immersion objectives of $\frac{1}{8}$th power and higher. The resolution into lines is not so difficult, when compared with that into dots. Mr. Wenham, when describing his new illuminator for diatoms, states that *he* was never successful in the patient manipulation required to resolve this diatom by the old methods of illumination.

Herr Möller produces what is called a "test-platte," containing 20 diagrams, mounted dry or in balsam; they are arranged upon the slide in a row, at the beginning and end of which is a specimen of *Eupodiscus argus*. The following is a list of the diatoms on this "test-platte,"

with the number of striations to the inch, as given by Morley :—

Diatom.	Number of Striæ to the Inch.
1. Triceratium favus	3,060 to 3,080
2. Pinnularia nobilis	10,800 „ 12,500
3. Navicula lyra	16,300 „ 18,500
4. „ „	25,000 „ 27,000
5. Pinnularia interrupta	26,500 „ 26,800
6. Stauroneis Phœnicenteron	31,100 „ 33,000
7. Grammatophora marina	36,300 ...
8. Pleurosigma Balticum	31,500 „ 34,300
9. „ acuminatum...	42,700 ...
10. Nitzschia amphyoxis	42,900 „ 45,300
11. Pleurosigma angulatum	43,800 ...
12. Grammatophora subtilissima	61,200 „ 61,700
13. Surirella gemma	51,400 „ 54,800
14. Nitzschia sigmoida	63,000 „ 63,300
15. Pleurosigma fasciola	54,500 „ 56,500
16. Surirella gemma	63,000 „ 70,400
17. Cymatopleura elliptica	63,300 ...
18. Navicula crassinervis	79,400 „ 82,200
19. Nitzschia curvula	84,500 „ 84,700
20. Amphipleura pellucida	92,700 „ 92,900

Mr. C. Fasoldt and Professor W. A. Rogers, in the United States, have produced ruled bands, comparable with those ruled by Herr Nobert, in Pomerania. We have not had one of Prof. Rogers' plates in our possession, but that of Mr. C. Fasoldt's, in our cabinet, does him much credit. Our plate is ruled with 18 bands as follows :—

I.	5,000	X.	50,000
II.	10,000	XI.	55,000
III.	15,000	XII.	60,000
IV.	20,000	XIII.	70,000
V.	25,000	XIV.	80,000
VI.	30,000	XV.	90,000
VII.	35,000	XVI.	100,000
VIII.	40,000	XVII.	110,000
IX.	45,000	XVIII.	120,000

The last band is ruled 50,000 lines to the inch, and is for facilitating the delicate adjustment of the objective; the cover glass is $\frac{5}{1000}$ths of an inch thick, the lower glass being $\frac{14}{1000}$ths of an inch in thickness.

Allusion has already been made to the variations likely o occur in the markings upon the frustules of diatoms nd the scales of insects. In order to avoid these irregularities, the late Herr Nobert, of Pomerania, issued a series f test lines ruled upon glass, each band containing lines of definite number to the inch. The most popular is that nown as the 19-band plate, containing lines to the inch as nder :—

Band.	Number of Spaces per Inch, about	Band.	Number of Spaces per Inch, about
I.	11,300	XI.	68,000
II.	17,000	XII.	73,000
III.	22,500	XIII.	79,000
IV.	28,000	XIV.	84,000
V.	34,000	XV.	90,000
VI.	39,800	XVI.	96,000
VII.	45,500	XVII.	101,000
VIII.	51,200	XVIII.	106,500
XI.	56,800	XIX.	112,000
X.	62,500		

Herr Nobert often expressed his opinion that the last our bands of this plate would never be resolved by any bjective; but after inspecting Dr. Woodward's photographs f the whole series, he produced another "plate" ruled to he twentieth band, the tenth on which corresponds to the ineteenth on the old, the twentieth band being said to be uled at the rate of 200,000 lines to the inch, though we are ot aware that any observer has resolved any higher band an the eleventh on this plate.

It has already been mentioned that the resolving power f objectives depends entirely upon their aperture, the xcellence of their corrections for colour and sphericity eing pre-supposed.

Objectives of low angle are generally made with posterior nses of such a size as to exclude the extreme uncorrected

marginal rays, or if this is not done the margins are cut off by a diaphragm. The utilised portion is very fairly corrected *for colour*. On the other hand, wide apertures require large back lenses, the margin of which cannot be cut off by a diaphragm without reducing the angle, and therefore the corrections have to be applied to more oblique pencils. It is this which makes them so expensive.

Competent microscopists have found that objectives of the widest aperture are very valuable for the study of the minutest and severest tests, and that they will stand deeper eye-pieces than dry lenses. Of course, the full performance of the increased aperture can be effective only when the object is mounted in a medium of a refractive index superior to the full aperture of the objective, or, if mounted *dry*, the object must be in absolute contact with the covering glass. If the object is separated from the covering glass by a film of air—no matter how thin—the objective will do no more work than a good water immersion of a numerical aperture of 1·0, or equal to 180° in air. The method of illumination must also be carefully attended to; the Bausch and Lomb immersion condenser is, we think, the most simple of its kind; but if expense be an object to be avoided, a nearly hemispherical lens cemented to the under surface of the slide, or Wenham's "glass button," will yield rays of any required obliquity.

A short time ago Professor Abbe introduced a new system of testing objectives, one based on more rational principles than any introduced before. The working distance, the penetrating power, and the resolving power of objectives being practically fixed quantities, that is, pre-supposing excellence of workmanship, and starting with a good formula for construction, it only remains to test the real excellence of that workmanship, and to find out whether the figure of the lens is correct, and whether the errors of sphericity and chromatism have been sufficiently cared for, and whether the

margin of the lenses have been as carefully corrected as the centres. Dr. Goring, in Pritchard's "Microscopic Cabinet," published in 1832, tells us how we may discover the presence of chromatic and spherical aberration by means of the globule of mercury test, a method used, we believe, up to the present day by most practical opticians for the final correction of lenses during the process of construction. These minute globules of mercury (called by Dr. Goring "artificial stars") are formed by crushing a globule of mercury with a sharp blow from a piece of steel clock spring, upon a slip of black glass, so as to form very minute spheres, reflecting the light of the sun, a lamp, or a picture of the window at the will of the observer. It would be excellent practice for the young microscopist, to make himself acquainted with the appearances of the "artificial star" under a $\frac{1}{4}$-inch objective of (110°) 0·82 N.A., varying the position of the correction collar and also the length of the body-tube of the microscope.

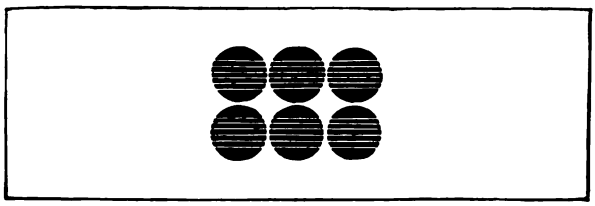


FIG. 146.

Much more scientific than the foregoing, and more accurate also, is the method for testing objectives devised by Professor Abbe. His test-plate (Fig. 146) is composed of a series of ruled bands on covers of varying thickness, which are as follows:—·09, ·12, ·15, ·18, ·21, ·24 millimeter. These covers are coated with a thin film of silver, and groups of lines ruled upon them; the ruled sides are then cemented with balsam to the polished slips. Each band is composed of ten lines ruled at about $\frac{1}{600}$ of an inch apart, and when

viewed with a good objective, presents a series of sharply defined black and white stripes, opaque and clear lines alternating at close intervals and lying absolutely in the same plane, so that no deviation can occur in the course of pencils of light transmitted through it.

A short and ready method of testing approximately any objective is recommended by Professor Abbe, as it is applicable to all instruments without requiring any apparatus except the test object already described. This may be briefly explained as follows:—

First, focus the test plate with central illuminating rays, then withdraw the eye-piece, and turn aside the mirror so as to give the utmost obliquity of illumination which the objective under trial will admit of. This will be best determined by looking down the tube of the microscope whilst moving the mirror, and observing when the elliptic image of light reflected from it reaches the peripheral edge of the field. As soon as this is done replace the eye-piece, and examine afresh the object plate *without altering the focus*. If the objective be perfectly corrected the groups of lines will be seen with as sharply defined edge as before, and the colours of the edges must, as before, appear only as those of the secondary spectrum in narrow and pure outline. Defective correction is revealed when this sharp definition fails, and the lines appear misty and overspread with colour, or when *an alteration of focus* is necessary to get better definition, and colours confuse the images.

A test image of this kind at once lays bare in all particulars the whole state of correction of the microscope, it being, of course, assumed that the observer knows how to observe, and what to look for.

In testing objectives by this method it is of great importance to use an ocular of high power, and to work with fairly brilliant illumination; and further, in employing

oblique rays, it is necessary that the light should be thrown in a direction perpendicular to the lines on the plate.

For critically testing objectives by means of isolated pencils, any suitable condenser, such as Abbe's, the "Webster," or the Bausch and Lomb may be employed in the ordinary rotative sub-stage fitting, diaphragms made of ferrotype plate or blackened cardboard being used to produce these pencils. If two pencils are to be employed, say when working with an objective, the front lens of which is $\frac{1}{4}$ of an inch in diameter, the image of the pencil of light entering should not occupy the space of more than $\frac{1}{16}$ of an inch. One of these should be made to extend from the centre of the field to $\frac{1}{16}$ on one side of the centre, the other should be made to touch the periphery of the field, so that there is a clear $\frac{1}{16}$ between its own inner margin and the centre of the field, as shown in Fig. 147. If three pencils of light be

FIG. 147.

FIG. 148.

employed, they should be arranged as shown in Fig. 148. The required size of these holes, which depends upon the focal length of the illuminating lens, as well as upon the aperture of the objective, may thus be found:—A test object being sharply focussed, a card diaphragm, having two or three holes in it, must be tried until that size be found the image of which in the posterior focal plane of the objective shall be about $\frac{1}{4}$ to $\frac{1}{6}$ part of the diameter of the field of the objective. Having thus found experimentally the required size of the holes, similar ones must be pierced in the diaphragm,

as already shown in Figs. 147 and 148, and placed *in situ* in the condenser. If the condenser is made to revolve round the axis of the instrument, carrying the diaphragm with it, the pencils of light admitted through the holes will sweep the face of the lens in as many zones as there are holes.

To examine an objective of wide aperture, each of the discs on the test-plate must be examined in succession, observing in each case the quality of the image in the centre of the field, and the variation produced by using central and very oblique illumination.

When the objective is perfectly corrected for spherical aberration for the particular thickness of cover-glass under examination, the outlines of the lines in the centre of the field will be perfectly sharp by oblique illumination, and without any nebulous doubling or indistinctness of the minute irregularities of the edges. If, after exactly adjusting the objective for oblique light, central illumination is employed, no alteration of the focus should be necessary to show the outlines with equal sharpness. If, on the other hand, every disc upon the test-plate shows nebulous doubling or an indistinct appearance of the edges of the lines with oblique illumination, or if the objective requires a different focal adjustment to get equal sharpness with central as well as oblique light, then the spherical correction is more or less imperfect. Nebulous doubling with oblique illumination indicates over-correction of the marginal zone; indistinctness of the edges without any marked degree of nebulosity indicates under-correction of this zone. An alteration of the focus for oblique and central illumination points to an absence of concurrent action of the separate zones, which may be due to either an average over-correction or under-correction, or to irregularity in the conveyance of the rays.

For chromatic aberration the best test is based on the character of the colour bands which are visible by oblique

illumination. With good correction the edges of the lines in the centre of the field should show only narrow colour bands in the complementary colours of the secondary spectrum—viz., on one side yellow-green to apple-green, and on the other, violet to rose. The more perfect the correction for spherical aberration the clearer this colour band appears.

To obtain obliquity of illumination extending for all distances from the centre to the marginal zone of the objective, Abbe's illuminating apparatus (or any other constructed in the same manner, such as that of Messrs. Bausch and Lomb) is very efficient, as it is only necessary to move the diaphragm in use nearer to, or further from, the axis, by the rack and pinion provided for that purpose. For the examination of ordinary immersion objectives of greater aperture than 1·00 N.A. it is necessary to bring the under surface of the test plate into contact with the upper lens of the condenser by means of a drop of water, glycerine, or cedar-wood oil as the case may be. In ordinary cases, the change from central to oblique light may be easily effected by the concave mirror; but with immersion objectives of wide aperture it is impossible to reach the marginal zone by this method, and the best effect has to be searched for after each alteration in the direction of the mirror.

It must be understood that the quality of the image outside the axis is not dependent on spherical and chromatic aberration, in the strict sense of the term. Indistinctness of the outlines towards the borders of the field of view arises, as a rule, from unequal amplification of the different zones of the objective. Colour bands in the peripheral portion—with good colour correction in the middle—are always caused by unequal magnification of the different coloured images. Imperfections of this kind, improperly called "curvature of the field," are shown to a

greater or less extent in the best objectives when their aperture is considerable.

In order to become acquainted with the appearances produced on the test plate by chromatic or spherical aberration, the author procured from Mr. Wray, the optician, some lenses of 1-inch and ½-inch focus, of crown and flint glass. These lenses only cost a few shillings each, and when used over the test-plate as an ordinary objective, give the student more experience of the effects of the two kinds of aberration than he can obtain from any other source.

CHAPTER VIII.

MICROSCOPICAL VISION.

PROFESSOR ABBE tells us "the very first step of every understanding of the microscope is, to abandon the gratuitous assumption of our ancestors, that microscopical vision is an imitation of macroscopical, and to become familiar with the idea that it is a thing *sui generis*, in regard to which nothing can be legitimately inferred from the optical phenomena connected with bodies of large size."

From the researches of Professors Helmholtz and Abbe it has been very clearly demonstrated that images of bodies of large size, when formed by lenses free from chromatic and spherical aberration, are in themselves geometrically correct pictures, truly resembling the objects themselves, were they enlarged to the same size and illuminated in a similar manner. Pictures formed in this way are termed dioptric images. When, however, the object is made up of particles less than $\frac{1}{2600}$ of an inch separated from each other, it is no longer possible to depict such structure by ordinary dioptrical means. The resolution of structures made up of particles having smaller intervening spaces than this is effected entirely by what is called "diffraction," the broad outline being, however, at the

same time depicted by dioptrical rays. The light in its passage through the object is modified, or scattered, and produces what are called diffraction spectra, which may be easily seen in the microscope when proper means are taken for their exhibition. If the ultimate particles of the structure are comparatively large, the diffraction spectra will most probably all lie within the field of the objective, whilst if they are small they may produce such a wide dispersion of the interference-spectra that only a part of them may range within the field, the remainder being lost, owing to the aperture of the objective not being sufficient to collect them. If only a portion of the diffraction spectra be collected by the eye-piece, it can scarcely be expected that the true structure is exhibited, and, in fact, this is the case, which can be easily exhibited by numerous experiments.

FIG. 149. FIG. 150.

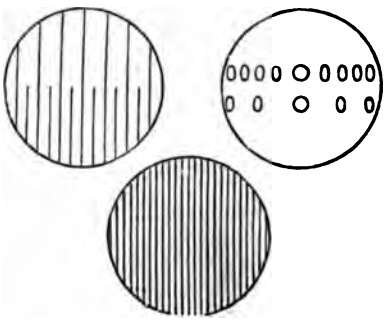


FIG. 151.

If a plate of glass with fine ruled lines, such as are represented in Fig. 149 in the magnified image, be placed upon the stage of the microscope, and a beam of white light from the mirror made to pass through a small opening in a diaphragm between the mirror and the stage, upon removing the eye-piece and looking down the tube, spectral images of the source of light will be seen on each side of the central

beam, as in Fig. 150; the closer images of the upper line being formed by the wider lines of the plate, and the more distant images by the closer lines. If a diaphragm be now placed at the back of the objective, covering all the spectra, and allowing only the central rays to pass, upon replacing the eye-piece the lines on the plate will have disappeared. If we now place another diaphragm at the back of the objective, so as to exclude all but the central rays and the outermost spectra, upon replacing the eye-piece we shall see more lines than are in the object, the appearance being as in Fig. 151. The false lines are the result of the phenomenon of interference or intermixture of the luminous waves. On this principle a great variety of effects may be produced from object-plates ruled to different patterns and an adequate manipulation of the spectra produced by them. Upon the admission or exclusion, more or less, of the diffraction rays, as they are bent off in angular groups, depends the capability of the microscope to show things as they are in nature, and therefore affects, not only the resolving power, but the delineating power of the instrument.

Professor Abbe says that very minute structural details, as a rule, "cannot be interpreted as morphological, but only as physical characters; not as images of material forms, but as signs of material differences of composition of the particles composing the object; so that nothing more can safely be inferred from the image as presented to the eye than the presence in the object of such structural peculiarities as will produce the particular diffraction phenomena on which the image depends."

In examining such minute structure as the striæ of a diatom, it has been shown that, if we take the distance of the striæ as $0{\cdot}6\mu$ (about $\frac{1}{42000}$ of an inch) and the wave-length of light as $0{\cdot}55\mu$ in the centre of the luminous spectrum, the first diffracted ray will be directed outwards, at an angle of

66°·5 from the axis, when central light is used, and this spectral ray will be collected on each side of the axis by a dry objective of 133° angular aperture, as in Fig. 152. In balsam the same rays will be deflected at an angle of 37½°, and will therefore be received by an objective of 75° balsam-angle. If the distance of the striæ be twice the former quantity, or 1·2μ, then the dry objective of 133° air-angle will admit two diffraction rays on each side the axis, while an immersion lens of 133° balsam-angle will admit three such rays on each side.

This is another proof that an angular aperture in air is not an optical equivalent of the same angle in balsam or oil, but that the latter is something more; and its capacity for showing things *as they are*, when the structural particles

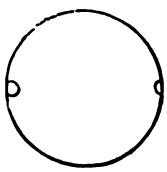


FIG. 152.

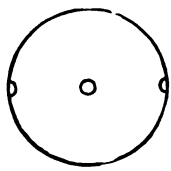


FIG. 153.

under investigation bear comparison in minuteness with the wave-lengths of light must be greater also.

As the structural elements become larger, the diffraction rays become contracted within smaller angles, until medium and low angles are capable of transmitting them, and the object at length becomes imaged upon common dioptrical principles.

If, instead of using central illumination, as in the last case, we now pass an *oblique* pencil of light through the smaller diatom, the direct ray and one spectral ray will be just received by an objective of half the angle of the former, or 66·5 in air, as in Fig. 153; and this aperture will be the theoretical minimum capable of separating lines=0·6μ. If

we want to see images more characteristic of the real structural elements than mere lines, we must use wider apertures. We may also diminish the wave-length of the light by employing rays corresponding with a part of the solar spectrum nearer the blue, and thereby gain the advantage of a slight decrease in the angle of diffraction. If the line E of the solar spectrum be taken, as in the numerical aperture table of the R. M. S., and the wave-length computed at ·5269μ, then the theoretical resolving power of an air-angle of 60° will be represented by 48,200 lines to an inch; and this power will increase in the direct ratio of the numerical aperture until the widest balsam-angle has been attained.

There is, perhaps, no theory more firmly established on a physical basis than the undulatory theory of light. If a wave of light cannot actually be seen, it can be *and has been measured.* According to Angström, the length of a wave of monochromatic light varies from ·7604μ, corresponding with the dark line A in the extreme red of the solar spectrum, to ·3933μ at the line H_2 in the extreme violet (1μ or micro-millimetre = $\frac{1}{1000}$ of a millimetre, or rather more than $\frac{1}{25000}$ of an inch); the line E near the centre of the luminous spectrum in the green having a wave-length of ·5269μ, or about $\frac{1}{48000}$ of an inch, which is about the distance of the striæ on *P. Angulatum.* So long as the visible details of an object are enough to be many multiples of the wave length of light, the rays are propagated from them in rectilinear directions, as in ordinary vision, or as seen through the telescope; but when these details are only a few multiples of the wave-length, the rays are intercepted by the minute elements of the structure, diffracted from their rectilinear paths, and result in *spectra*, or distorted images.

For a long time opticians despaired of being able to improve the optical portion of the microscope, and in the year 1873 it was openly expressed by many that improve-

ments had been pushed as far as it was possible to go. It was known, however, that the secondary spectrum still remained uncorrected; but the suppression of this error was practically considered impossible; many had been the attempts which had been made, and all had ended in failure.

Within the last few years, however, the suppression of the secondary spectrum has been accomplished by the united labours of Prof. Abbe, Dr. Otto Schott, and Herr. Carl Zeiss, all of Jena. Dr. Otto Schott is an able chemist, who has had much experience in the manufacture of glass, and since 1881 has been experimenting upon the improvement of so-called optical glass, the result of which has been the establishment of a glass factory at Jena, to supply all kinds of glass for general optical purposes. New materials have, therefore, been placed in the hands of opticians, and, no doubt, many uses will be found for this glass other than that which forms the subject of the present notice.

As already stated, complete achromatism had, up to this time, been unattainable on account of the disproportionate dispersion at different parts of the spectrum, and also it had not been found possible to correct the spherical aberration for more than one colour. By use of the new glass these two errors may now be practically eliminated. It is true, however, that, theoretically, there is a residuary aberration still to be suppressed, but the great point gained is, that the chromatic difference of spherical aberration can be practically eliminated, or, to speak more plainly, the spherical aberration can be corrected for two different colours simultaneously, and practically so for all colours.

This has been accomplished by altering the chemical composition of the glass, in which phosphates and borates have been introduced, the former in crown, and the latter in flint, and the Glastechnisches Laboratorium, of Jena, issue a circular of the optical properties of several kinds of glass

manufactured, thus supplying the requisite data for their application to the construction of objectives.

By the practical suppression of the secondary spectrum, the full aperture of the objective may now be utilised, and these lenses have been called by Professor Abbe, *apochromatic.* They may be used with a much deeper eye-piece than ordinary objectives, without sensible loss of definition, so that a very great degree of amplification may be gained by the use of objectives of moderate power and fair working distance. It has been already mentioned that a residuary aberration still exists—it has been found that in all objectives of large aperture there is a certain amount of colour deviation outside the centre of the field; this is caused by the fact that the image formed by the blue and violet rays is really larger than that formed by the red and yellow. This error exists also in the new apochromatic objectives; but, fortunately, and unlike the objectives of older construction, the aberration can be readily corrected by means of the eye-piece.

For the purpose of making the image appear as perfect as may be when using the apochromatic objectives, a special ocular is employed, which is styled by Herr Zeiss the compensation ocular, which behaves as a strongly over-corrected lens. Moreover, the construction of these eye-pieces is so different to those in ordinary use, that considerable advantages are gained in their adoption. It is well known that with the present Huyghenian oculars of high power the focal point lies very close to the surface of the eye-lens, so close, in fact, that the ordinary camera lucida cannot be employed with them. In the compensation ocular, the diameter of the eye-lens is considerable, and the focal point so far from the lens that the camera lucida may be used with the whole series. Searcher eye-pieces have also been made in this series, yielding with every objective exactly that magnifying power which the objective would yield alone if used as a

lens without any eye-piece. The series of oculars yield amplifications from 1 to 18, so that a homogeneous immersion objective giving, *per se*, a magnifying power of 80, would produce 1,500 with the strongest ocular. Professor Abbe is of opinion that homogeneous objectives of smaller focal lengths than 3 mm. will become superfluous, and the same may be said of dry objectives below 4 mm.

It must not be forgotten that an ocular can only have a magnifying power varying with its distance from the objective, and therefore, although Zeiss designates his new eye-piece by a number expressing the super-magnifying power which they give to the microscope, the tube length must be definite and constant for these numbers to be accurate.

In the set of apochromatic objectives as constructed by Zeiss, the apertures rise from 0·3 to 1·4, and it has been stated that in each class, *i.e.*, dry, water-immersion, and homogeneous-immersion, the theoretical maximum of aperture has been realised to within 7 per cent.

Messrs. Powell and Lealand are now constructing apochromatic objectives and compensation oculars from Jena glass, which are admirable specimens of optical art: the $\frac{1}{8}$th, $\frac{1}{10}$th, and $\frac{1}{12}$th possess a numerical aperture of 1·40, and are charged at £25 each.

CHAPTER IX.

THE COLLECTION OF OBJECTS.

IT has been the author's endeavour to persuade the student to take up some special branch of study with the aid of the microscope, and with this end in view the following chapter has been written, showing where certain objects are to be found, what apparatus is requisite for their collection, what to collect, how to collect, and when gathered, how to preserve them for future examination under the microscope.

Collectors of experience will not require to be informed on many of these points, and therefore to make the chapter interesting to more than the mere student, a list of works treating on each subject has been appended, and also the names of several species under each heading, in order that the possessor of a microscope might know what slides to purchase, should he desire to fill a cabinet in that manner.

The author would strongly advise the young student to refrain from flitting hither and thither over the whole range of microscopical objects. It is not enough to be able to name a few rotifers or rare diatoms, such knowledge is of the shallowest kind; but if he sets himself to work to study the life-history of some hitherto obscure organism, or the anatomy of an insect, the outward form of which he is alone

familiar with, he may rely upon it he will be useful in his generation.

Most collectors have their own method of gathering specimens, and are very conservative on this point, but the telescopic walking-stick with all its fittings, as shown in Fig. 154, is an article generally used by all. There are generally

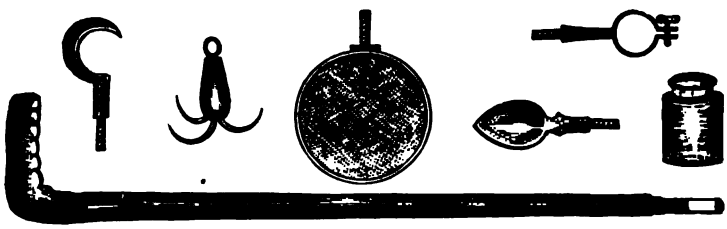


Fig. 154.

supplied with it, a ring to carry a fine muslin net, a ring to held a bottle, a weed knife, a spoon, and a drag hook for weeds.

Mr. Baker, of Holborn, supplies the article in rather a different form: the bottom ring is not clamped by a screw, as shown in the figure, but is furnished inside with a thread, into which is made to screw the neck of one of the York Glass Co.'s bottles.

For many purposes a pond scoop is required, such as for scraping the surface of the mud at the bottom of pools when searching for Oscillatoria, &c., and if it is made to screw into the end of the collecting stick, it will be very convenient. It is simply a ring of tinned iron about 5 inches in diameter and 1 inch deep. Both edges are "wired," as the tinsmiths call it, so that a piece of thin muslin or stout gauze may be stretched tightly over it. It is shown in Fig. 155.

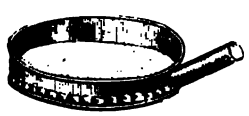


Fig. 155.

Some collectors prefer to secure the muslin over the scoop with a firm elastic band, so that after collecting, it may be removed and folded up for transport home—and perhaps this is the better plan.

The tow-net, Fig. 156, is of great use in collecting marine, and even river or lake objects. It is made of fine but strong muslin, tied at the large end round a wooden hoop, while the nethermost extremity is secured round a small wide-mouthed bottle, so that the more delicate organisms may find their way into it, and so be out of the way of the currents caused by the passage of the net through the water.

Fig. 156.

The tow-net, as illustrated, is furnished with an interior net, which, acting as a valve, prevents the escape of organisms which have once been enclosed.

Aquatic organisms, whether animal or vegetable, are met with in all kinds of water; even the tap water supplied by some of our corporations is extremely rich in specimens, while in clean ponds the collector will not fail to find a host of treasures; in impure streams and pools, containing sewage and other decomposing matters, only such common animalcula as *Paramecium aurelia* are to be found. Other objects are fixed upon stones and weeds under water, and little pieces of dead stick are often found covered with interesting objects.

When the water is not rich in specimens, it may be

necessary to concentrate them by straining off the superfluous water, which may be effected by using the filter shown in Fig. 157. It consists of two small funnels passing through a cork, as shown in the figure, the one which is inverted in

FIG. 157.

FIG. 158.

the bottle being covered at the mouth with very fine muslin. The water containing the organisms is poured in at the top funnel, while water only issues from the stem of that inverted in the water.

This operation may be continued until the bottle is well tocked, when the contents may be carried homewards for xamination.

When an organism is required to be removed from a ottle of water, a tube or tubes of the form shown in ig. 158 will be found necessary. They may be cut from rdinary glass tubing by making a cut with the edge of a hree-square file, and breaking it in two with the fingers. he sharp edges should then be fused by holding in the ame, finally allowing to cool gradually. The bent tubes ay be made by taking a length sufficient for two tubes nd softening the middle portion in the flame of an rdinary gas burner or spirit lamp, and when sufficiently

FIG. 159.

oftened the two extremities are to be pulled asunder so as o form a couple of tubes of the form of B, Fig. 158. They an then be cut asunder with the file and the edges fused. he form A is produced in a similar manner, the softened ortion being drawn out obliquely.

It is a great mistake to load oneself with a host of araphernalia. The labour of carrying a heavy pack often estroys what might otherwise have been an enjoyable xcursion.

A set of half-a-dozen small corked bottles or tubes, and

as many small tinned boxes, will complete the collector's outfit. A small but handy pocket collecting case was introduced several years ago by Stanley, of London. (See Fig. 159.)

The objects having been collected, the next thing is to find out what they are, genus and species, often no easy task for the beginner.

One way of getting over the difficulty is by consulting the books mentioned under each heading—nearly always to be had at the free libraries—often a long, laborious, and unsatisfactory task; while the other is by sending the specimens in a tube to a friend or naturalist of repute. In the event of one not being known, the author has much pleasure in suggesting the name of Mr. Bolton, jun., whose father established a naturalist's studio in Birmingham many years ago.* A word to such inquirers—never forget to enclose a stamp for reply: many forget this, and thus the willingness of the naturalist to furnish information gratis become a serious tax upon his pocket.

We may now pass on to the enumeration of many objects of interest; but let it not be for a moment supposed that it is possible to give a complete list of objects suitable for microscopic study. The main wish of the author is, to put before the reader something he may collect easily, in the hope that he will become interested in the study of the details of some one of them.

ALGÆ.—The members of this class of Thallophytes may be found almost anywhere, in ditches, streams, ponds, and even in the small pools of water lying in the hoof-prints of animals upon clayey or boggy soils. One of the most interesting of the Algæ is the *Volvox globator* (Fig. 160), which, however, is very uncertain in its habitat. Wherever

*57, Newhall Street, Birmingham.

found it is usually plentiful. All the fresh-water Algæ may be collected by the use of the appliances already mentioned. Many Oscillatoriæ grow upon the surface of the mud at the bottom of pools, and so require the scoop shown in Fig. 155. The whole collection, including mud, should be wrapped up in the muslin, and carried home in that state for examination. The remainder of the Algæ may be carried home in tubes or bottles, and upon arrival should be emptied into small aquaria formed of wide-mouthed bottles or small propagating glasses turned upwards, the knob resting in a hollow support. Whether minute or not, the gatherings should be examined on the spot with a platyscopic lens, to prevent the loading of one's satchel with useless specimens.

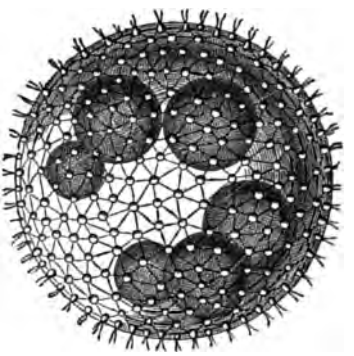


FIG. 160.

Marine Algæ furnish many beautiful objects for the microscope, and can be easily collected upon many shores. Perhaps of all places, Tenby, Torquay, and the Mumbles, near Swansea, are the best hunting grounds. The various species of Cladophora, Ptilota, Dasya, Bangia, Ceramium, and Griffithsia all form good objects.

Books which may be consulted: Rabenhorst's "Flora Europæa Algarum"; Griffiths and Henfrey's "Micrographic Dictionary"; Johnstone and Croall's "British Sea-Weeds" Hassall's "Fresh-Water Algæ."

ANIMALCULES.—Taking this term to apply to the Infusoria and Rotatoria, the student will find a good field for study. It is scarcely possible to find a drop of water, which has been for any length of time exposed to the air, not containing either

Infusoria or Rotatoria. In some waters they are found in but few numbers, while other localities literally swarm with them. In the former case the pond-filter, shown at Fig. 157, will be found valuable. The organisms may be taken from the pond or stream by means of the stick and bottle, and after straining, the residual water carried home in the cork tubes or bottle which the collector may have with him. A word of advice to the student: Do not overcrowd the organisms, and do not leave any portion of the bottle or tube filled with air if they are to be exposed to shaking or concussion. On arriving home the contents of the tubes may be emptied into small aquaria improvised from broken wine glasses, or—better perhaps—the tube can be stuck through the centre of a large cork and floated in a vessel of water to maintain an equal temperature, when the organisms can be easily abstracted as required by means of a dipping tube.

Fig. 161.

Amongst the Infusoria the *Euglena viridis*, *Paramecium aurelia*, and *Coleps hirtus* are good objects for study; of the Rotatoria, *Anuræa longispina* has been found in the tap water furnished by the Birmingham corporation, and others are to be met with in the same habitat, notably *Triarthra longiseta* and *Salpina reduncа*.

Melicerta ringens, the tube-building rotifer (Fig. 161) is a beautiful object, and should be carefully searched for; it is frequently found attached to water plants, such as the *Potamogeton crispus*, or large-leaved pond-weed; the *Anacharis alsinastrum* and *Myriophyllum spicatum*.

Books which may be consulted: W. Saville Kent's "A Manual of the Infusoria"; Griffiths and Henfrey's "The Micrographic Dictionary"; Pritchard's "History of the Infusoria."

ARACHNIDA.—This class of animal life, containing the spiders, may become very interesting and instructive objects. It seems hardly necessary to say where they may be found, or how to collect them; but it may be necessary to enjoin keeping them moist when they are required for permanent objects or for dissection; diluted glycerine or diluted acetic acid will effect this.

The respiratory system, the circulating system, the spinning organs, and even the eggs, are very interesting; but a knowledge of dissection must be gained before the student can make a successful study of this branch.

The Arachnida are very plentifully distributed—the mites or Acarini, such as the *Acarus domesticus* (cheese mite) and the *Acarus farinæ* (flour mite) are good objects for the ½-inch objectives used binocularly, either alive or when mounted without pressure.

Books which may be consulted: "Micrographic Dictionary"; Blackwall's "British Spiders" (Ray Society); Walker's "British Spiders" (Ray Society).

ANIMAL PREPARATIONS.—The number of these objects is legion; and little else can be done here than to say that animal preparations, as a rule, require special preparation and treatment. Still there is the raw material to collect, and this should be carefully preserved, in order that, when examined, its characters shall be faithfully delineated.

The hairs of animals and scales of fish present no unusual difficulties; but such subjects as skin, tongue, liver, lung, &c., should be reserved until the student has become a moderately expert experimentalist. Frogs, mice, rats, rabbits, and guinea-pigs are generally pressed into this service.

Books which may be consulted: "How to Work with the Microscope," Beale; Brunton, Foster, Klein, and Sanderson's "Handbook for the Physiological Laboratory"; Sylvester Marsh's "Section Cutting"; Rutherford's "Practical Histology."

CRUSTACEA.—In this branch are specimens innumerable,

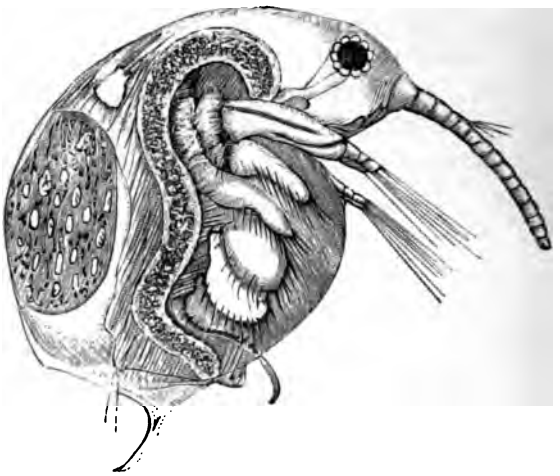


FIG. 162.

the Entomostraca being included under this head. They may all be taken with the appliances already mentioned, and what will do for Infusoria will also be sufficient for Crustacea. *Daphnia pulex*, the water flea; *Cyclops quadricornis; Cypris tristriata; Argulus foliaceus*, the fish louse; *Asellus vulgaris*, the water wood-louse; *Gammarus pulex*, the fresh-water shrimp; *Bosmina longirostris* (Fig. 162); *Chirocephalus*

diaphanus, the fairy shrimp, are all to be found in easily accessible ponds during the spring, summer, and autumn.

Books which may be consulted: Baird's "British Entomostraca"; "The Micrographic Dictionary."

DIATOMACEÆ.—This probably has been the most attractive class to nearly all microscopists. Diatoms are a family of Confervoid Algæ, in which the protoplasm is enclosed in silicious valves, generally covered with very fine markings, the nature of which has not yet been satisfactorily made out. They are found in fresh, brackish, and salt water, adhering to plants and stones, or scattered amongst peat, water mosses, or Oscillatoriæ, and even upon damp ground. Nothing is easier than their collection, but, of course, it is not always possible to meet with the specimens desired.

Diatoms are often found in the stomachs of fish, especially crustaceans and molluscs, and several species have been found in the internal arrangements of *Noctiluca miliaris*, a small exceedingly transparent organism of the size of a grain of mustard seed, causing a phosphorescence in the sea.

A little experience will enable any one to find and to gather all he may desire. Those living in the city can easily procure many beautiful varieties, by simply fastening a muslin bag like an umbrella cover to the hydrant. After securing a quantity of the sediment, empty it into a large fruit jar or other receptacle nearly filled with water, and let it settle.

The green, brown, or fawn-coloured scum on the surface of pools, bogs, and marshes, is mostly diatoms, and it may be taken up by means of a spoon or bottle and preserved, always in alcohol and water, or dried upon paper. The living weeds should be taken carefully from their location without much compressing or washing. The finer water plants yield the richest harvest. Fresh-water forms are sometimes found hanging in green-coloured masses from drains, sluices, and water-pipes. To gather from the lake, a net of fine muslin,

having an opening in the bottom, in which a wide-mouthed phial is tied, may be towed at the stern of a steamer. The sediment left in the bottom of pails, barrels, and other vessels contains a good supply. To obtain varieties not found at home, open a correspondence with gatherers in other localities who will gladly exchange.

In the collection and recognition of diatoms, the student will find Professor Brown's pocket microscope a useful adjunct, as it is furnished with a deep eye-piece and objectives of an inch, and a fifth of an inch focus. It is shown in Fig. 163.

FIG. 163.

Great care should be taken in the collection of diatoms, so as to have them in as pure a state as possible, as it is not easy to separate them from foreign matter when it is mixed up with them. The late Dr. Redmayne placed the gathering in a long bottle in the sun for a few hours, the lower half of the bottle being covered with black paper. The free diatoms separate themselves from the mud and come to the surface, and can thus be removed.

Fossil Diatomaceæ also exist in immense quantities in various places. The large deposits of guano, the Bermuda deposits, the Berg-mehl in Norway, the Mourne Mountain deposit in Ireland, the recent discovery of diatoms in the London clay by Mr. Shrubsole, and the still more recent discovery in Llyn Arenig Bach, about midway between Bala and Festiniog, in North Wales, show that the Diatomaceæ are, and have been, very widely distributed.

Some collectors may not consider the pocket microscope

shown in Fig. 163 steady enough for general use, and therefore may prefer the form shown in Figs. 164 and 165, as made by Mr. Browning; it is exceedingly portable and very

FIG. 164.

steady, as the author can testify from its practical use. The stage is fitted with rectangular and circular motions, has an adjustable sub-stage with centering movements, and is fitted

with plane and concave mirrors, on jointed arm. When fitted up, as shown in Fig. 164, it is as steady as a stand of the ordinary make, and by a novel arrangement the body of the microscope turns on a joint, and packs away into a case, the outside dimensions of which are 6 by 6 by 9 inches; when folded up for packing it appears as in Fig. 165.

Amongst the diatoms which may be singled out for examination are *Pleurosigma angulatum* (Fig. 143), *P. formosum* (Fig. 142), *Navicula firma* (Fig. 166), *N. lyra*

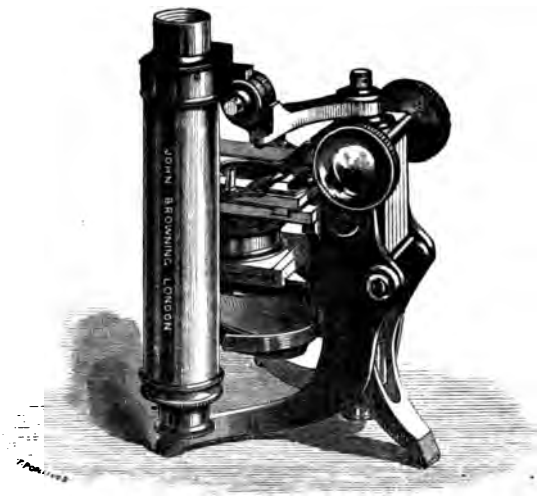


FIG. 165.

(Fig. 167), *N. rhomboides* (Fig. 144), *Isthmia enervis*, *Arachnoidiscus Ehrenbergii*, *Meridion circulare*, *Diatoma vulgare*, and a host of others.

Whilst writing this chapter the author has received a tube of diatoms from Mr. Bolton, of Birmingham, consisting of a number of species found attached to algæ in the canal of that neighbourhood; the most easily recognised were:— *Bacillaria paradoxa*, *Nitzschia sigmoidea*, *N. lanceolata*,

Grammatophora marina, *Amphiprora alata*, *Pinnularia radiosa*, and *P. viridis*.

Books which may be consulted: Rabenhorst's "Flora Europæa Algarum"; William Smith's "British Diatomaceæ"; Catalogue of the Diatomaceæ, Habirshaw (now publishing); and Schmidt's "Diatomacean Atlas."

ECHINODERMATA.—The marine objects, star-fishes, sea-eggs, or sea hedgehogs, may always be taken at low water after a spring tide. In their earlier stages they are extremely

FIG. 166.

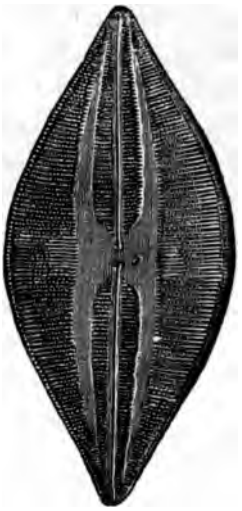


FIG. 167.

interesting, being infusoria-like organisms, and often appearing without any internal structure whatever. They furnish the microscopist with abundance of material. The prickles or spines, hooks, and the pedicellaria make interesting and instructive objects.

A section of Echinus spine is used as a test for the flatness of field in low-power objectives, while a section of a small spine may be used for the same purpose with higher

powers. A section of one of these may be seen delineated in Fig. 138.

Books which may be consulted: Forbes's "British Star-Fishes"; Agassiz's "Echinodermata Viv. et Foss."

FERNS require no special apparatus for collecting save a sharp knife and a tin collecting box; they form a very interesting study. The stem may be double stained, as described in the chapter on the staining and injecting of objects.

How many microscopists are there who possess *fern preparations* in their cabinet, spores and sori, stems in cross and vertical section, double and single stained, and yet are totally unacquainted with the life-cycle of a single species; who have looked at sori and spores innumerable, and yet never made the effort of allowing these to germinate, and to observe them in their various and strange mutations.

The *Lastrea filix-mas*, or male-fern, is one of the best species to study for the beginner, and there are many others easily found, such as *Athyrium filix-fœmina* (lady fern), *Scolopendrium vulgare* (hart's tongue), *Pteris aquilina* (common bracken), *Adiantum capillus-Veneris* (maiden-hair), and many others.

Books which may be consulted: Moore's "Index Filicum"; "Handbook of British Ferns"; Newman's "British Ferns"; J. Smith's "Ferns, British and Foreign"; Hooker and Baker's "Synopsis Filicum."

FORAMINIFERA.—These gelatinous, structureless animals are mostly sought after for the sake of the shells, serving them as a covering. The shells are pierced with holes, through which the animal protrudes its pseudopodia, using them as a means of locomotion. They are found in largest numbers in the sand and mud from the sea-bottom, but may also be found on sea-weeds, and in the fossil state in chalk, limestone, and other mineral deposits.

n sea-soundings they may be procured by dissolving out callow in which they are collected, by means of benzine—sort of benzine or benzoline serving this purpose. To in fossil Foraminifera from chalk, the pieces must be en up small by means of a hammer, and then gently hed in an iron mortar. The powder is then to be placed piece of coarse calico, tied up like a pudding, and put a large basin of water, and well kneaded until reduced ne-third its original bulk. The milky fluid is then to be ed off, until only one-fourth remains, and the operation ashing, by stirring up with fresh water, and allowing to e, repeated many times, until a portion of the residue, on nination under the microscope, shows that most, or all, of extraneous matter has been eliminated.

Amongst others, *Lagena squamosa*, *Orbitolites complanatus*, *stomella crispa*, and *Nodosaria raphanus*, are very good ts.

Books which may be consulted: Williamson's "Recent minifera" (Ray Society); Carpenter's "Introduction to Study of Foraminifera."

FUNGI.—Micro-fungi may be found everywhere, and e a splendid study. Many of them, however, can only examined successfully when in the fresh state, such as *cillium crustaceum* and *Aspergillus glaucus*, the common lds and mildews of our houses. Nearly every plant and is attacked at one time or another by some particular ies of micro-fungus, so that the student will find plenty ork in this class alone. We have the *Puccinia graminis* the leaves and stems of standing corn, as well as *Tilletia s* and *Ustilago segetum*, or smut, which fill up and destroy whole contents of the ear; the *Æcidium* on the berberry ; *Triphragmium* on the leaves of the meadow-sweet; the berry brand, *Aregma bulbosum; Coleosporium synan-rum* on the colt's-foot; *Cystopus candidus*, on cabbages;

Peronospora infestans on our potatoes; *Peronospora gangliformis* on lettuces; and *Peronospora viciæ* on peas: a host of others being within easy reach.

The micro-fungi, shown in Fig. 168, are as follow:—

a. Stachybotrys lobulata.
b. S. atra.
c. Penicillium nitophilum (Oidium aurantiacum).
d. Myxotrichum deflexum.
e. Polyactis fasicularis.
f. Sporocybe alternata (Aspergillus alternatus).
g. Rhopalomyces pallidus.
h. Papulaspora sepedonioides.
i. Acremonium alternatum.

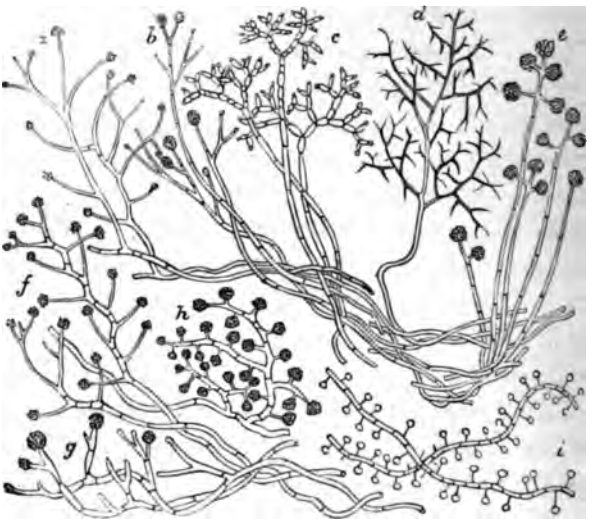


FIG. 168.

They have all been found upon moist cotton goods or in analogous situations.

All that is necessary in collecting micro-fungi is to well isolate each specimen, preferably by wrapping in soft paper, and placing separately in a small box.

Books which may be consulted—"Outlines of British Fungology," by Rev. M. J. Berkeley, F.R.S.; "Handbook of

sh Fungi," by M. C. Cooke; "Rust, Smut, Mildew, and d," by M. C. Cooke; "Selectæ Fungorum Carpologia," s., Tulasne.

FUSORIA AND ROTATORIA.—ly all that is necessary to say r this head has been given treating of animalcules. h-water infusoria may be col-l in wide-mouthed bottles, and ndividuals selected for exami-n removed by means of one ıe dipping tubes shown in 158. The members of this of animals are generally to be l attached to weeds. The tiful *Stephanocerus Eichhornii* e of this class; it is shown in 169. The author has before at the present moment some ts of *Myriophyllum spicatum*, ɔmmon water-weed, completely ed with *Melicerta ringens*, *ularia cornuta*, *Philodina me-rocha*, *Limnias ceratophylli*, *lanis dilata*, and *Codosiga tis*. Most of these organisms be obtained from Mr. Bolton's ɔ.

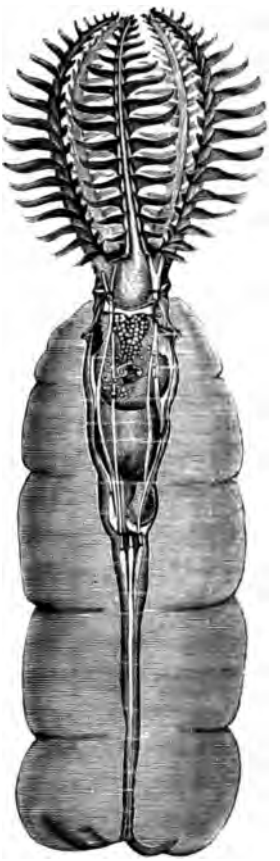


FIG. 169.

ooks which may be consulted Animalcules).

NSECTS afford an inexhaustible series of treasures to the ɔscopist; but, in order to examine these perfectly, a ledge of dissection must be gained. The head, eyes, legs, s, proboscis, spiracles, and tracheæ, all make instructive

objects. Many are the kinds of insects that can be pressed into the microscopist's service, the *Siphonoptera*, or fleas; the *Parasitica*, or lice; the *Diptera*, or flies; the *Hymenoptera*,

FIG. 170. FIG. 171.

bees and wasps; *Lepidoptera*, butterflies and moths; *Orthoptera*, grasshoppers and crickets; the *Coleoptera*, or beetles, are all too well known to require instructions when, how, and where to collect.

FIG. 172.

Fig. 170 shows the male *Dytiscus marginalis*, or great water beetle, a very interesting insect to the microscopist, while Fig. 171 is a picture of the female.

The larvæ too are an interesting study; this stage in the gnat, dayfly, and even of the *Dytiscus*, Fig. 172 will amply repay the observer for the attention he bestows upon it.

When required for immediate examination, no special

care is requisite; but if for dissection and permanent mounting afterwards, they should be immersed in a mixture of equal parts of glycerine and water, dilute acetic acid, or even dilute alcohol (for some things which require hardening), in order to procure preservation. It must be understood,

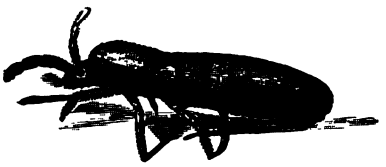


FIG. 173.

however, that this is not universally applicable. There are some objects which would be spoiled by immersion in a fluid, and these, of course, must be prepared while in the fresh state.

The scales of several insects are much used as test objects, notably those of the *Lepisma saccharina*, *Morpho menelaus*,

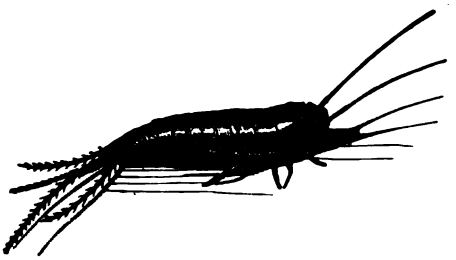


FIG. 174.

the *Polyommatus argus*, the *Lepidocyrtus curvicollis*, and *Podura plumbea*. These scales are shown in Chapter VII. The *Podura plumbea* is a small insect found in damp shady places, under stones in cellars, where they may be caught by sprinkling a little oatmeal near their haunts. They are commonly called springtails; and in order to help the student in finding them, an illustration is given in Fig. 173,

and of the *Lepisma saccharina* in Fig. 174, each magnified about 12 diameters.

FIG. 175.

The Ephemeridæ or May-flies form a very good study. A paper appeared in the "Northern Microscopist," in 1881, from the pen of Mr. W. Blackburn, to which the reader is

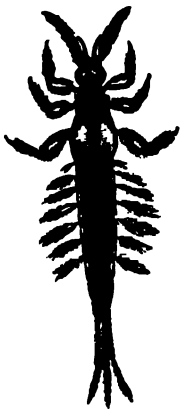


FIG. 176.

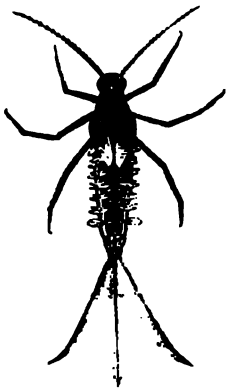


FIG. 177.

referred. The image of the *Ephemerica danica* is shown in Fig. 175; it is the ordinary May-fly of the angler. They have been seen on the river Dove in such numbers as to almost cover the water where the stream was from twenty to thirty feet broad. Fig. 176 is an illustration of the nymph of the Ephemera, while Fig. 177 sets forth very accurately the appearance of the nymph of the *Clöeon dipterum.*

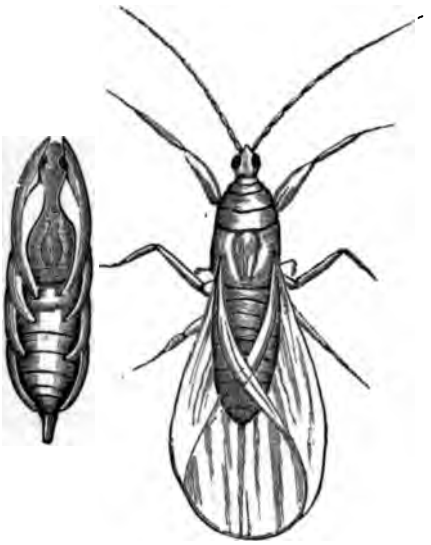


FIG. 178.

The microscopist may sometimes find many specimens of interest without going out of doors. The rind of the orange is often infested with a species of Coccus, the oranges from St. Michaels being the most prolific in these specimens. On their surfaces the various stages of development may be easily studied, from the egg to the perfect male and female. It is called the *Tecamium Hesperidum*, and is shown as the perfect male insect in Fig. 178.

It is very interesting at times to watch the development of insects and the changes they undergo during their cycle of existence; for this purpose a "vivarium" is necessary, so that while furnishing them with sufficient air they may be prevented from crawling or flying away, and the respired carbonic acid find easy exit.

The best kind of vivarium is made of perforated zinc, with a bottom of the same material, on which rests an inner circle of zinc but perforated more closely. This is not fastened to the bottom, but can be raised at will; it is covered with a round piece of flat glass. On this stands a saucer of well moistened clay stuck round the edge with such leaves as the insects feed on. In the centre of the saucer is a small vase filled daily with fresh flowers and grasses for the butterflies.

It is easy in this apparatus to raise moths, butterflies, and other winged insects, such as saw-flies and the like, from the egg; and the many changes may be watched through the glass, from the emergence of the caterpillar from the egg, to the exit of the imago from the pupa case.

LICHENS.—These interesting vegetable productions require but an old knife and a few pill-boxes for their collection, with occasionally a hammer and chisel. The purer the atmosphere of the district, the greater variety to be found, while in a smoky and gas-polluted atmosphere, they are either not found at all, or only to be met with in the gonidial state, when they may be easily mistaken for protophytal algæ. Lichens are to be found growing upon rocks, stones, trees, old railings, twigs, bushes, heath, moss, and in many other places.

Lichens are mostly of large size, and require thin sections to be cut ere the structures can be satisfactorily made out; but some should be always mounted as opaque objects, to show what the natural form of the plant is.

Fig. 179 shows a piece of fencing, upon which is growing

Lecanora cerina, while the next illustration, Fig. 180, shows *Usnea barbata*, one of the filamentous species. Among the more familiar kinds are *Parmelia parietina*, *Lecidea canescens*, *Physcia ciliaris*, *Cladonia pyxidata*, and *Pertusaria communis*.

Books which may be consulted:—"History of British Lichens," W. L. Lindsay, M.D.; "Lichenes Britannici,"

FIG. 179.

FIG. 180.

Crombie; "The Lichen Flora of Great Britain and Ireland," Rev. W. A. Leighton.

MINERALS.—Of late years, much has been discovered in the mineral kingdom by the aid of the microscope, but owing to the nature of the substances to be examined, much preparatory work has to be done ere a satisfactory examination

can be made. Sections have to be cut and semi-polished ere the structure can be well made out, and this will be described in our next chapter. Barbadoes rock, showing Polycistina *in situ*, oolitic and nummulitic limestone, slags from iron and copper furnaces, fossil wood, minerals used in smelting, basalt, boiler incrustations, coal, shale, lava, and many other minerals, furnish instructive objects, and may be collected in plenty in the proper localities.

FIG. 181.

The two figures, 181 and 182, show the artificial production of crystals during the manufacture of glass. When

FIG. 182.

the "metal" is cooled slowly, crystals of certain forms are obtained, which vary in form according to the differences existing in the quantities of the materials from which the glass is made.

Books which may be consulted:—"Sur les Crystallites," Vogelsang; Allport on the "Microscopical Examination of Rocks and Minerals," p. 98, "Monthly Microscopical Journal;" "Mineralogy and Study of the Rocks," Rutley.

MOLLUSCS.—The entire series of the Mollusca form very interesting objects for the microscope, the structure of the viscera, the spermatozoa, and the tongue, or *odontophore*, being the chief. Fresh-water and land snails should be avoided by the student until he has gained sufficient experience in the manipulation of limpets and whelks, from which the extraction of the *odontophore* is a comparatively easy operation. The marine molluscs may be carried long distances folded in damp sea-weed, while fresh-water snails should be conveyed in a similar manner in damp Anacharis. When the animals are not immediately required, they may be dropped into glycerine, dilute acetic acid, or into alcohol. Edwards advises that they be dropped into caustic potash solution (say one part of caustic potash and two of water), until they begin to decompose; but of course this is only admissible when nothing but the *odontophore* is required.

This odontophore is the so-called tongue or lingual ribbon, really the masticatory apparatus, a long ribbon-like organ furnished with a complicated system of teeth, generally set upon flattened plates. The form and arrangement of the teeth furnish characters of much importance in classification, and therefore should be studied by all those who are interested in this department of natural history.

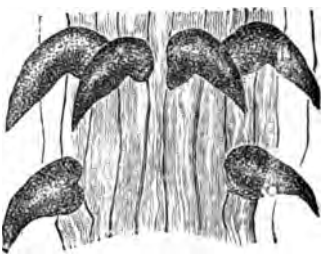


FIG. 183.

Fig. 183 shows a short length of the odontophore of the common limpet, *Patella vulgata*.

Books which may be consulted:—"British Mollusca," Forbes and Hanley; "Notes on Victorian Molluscs and their Palates," C. M. Mapleston, "Monthly Microscopical Journal," vol. viii., p. 45; Alcock, "Proceedings of Manchester Literary and Philosophical Society."

MOSSES.—These require but few instructions for collecting. They are of little use for the purpose of scientific inquiry without fruit, and therefore the collector should always endeavour to procure the plant in fructification. *Polytrichum commune*, *Sphagnum squarrosum*, *Hypnum purum*, *Tortula* (*Barbula*) *muralis*, are amongst the more common species.

Every month produces some species to be collected; on old walls, the roofs of tiled and thatched buildings, on our garden walks, on our downs and commons, on chalk and sandstone, in bogs and on the mountain-top, mosses may be found everywhere. The necessary apparatus for collecting consists of an old pocket knife, a platyscopic lens, as shown in Fig. 2, a satchel or small bag, and a good supply of thin envelopes, or square pieces of tissue or other thin paper, in which to wrap each species.

Books which may be consulted:—Hooker, Taylor, and Wilson's "Bryologia Britannica," R. Braithwaite's "British Moss Flora," Stark's "Popular History of British Mosses," Berkeley's "Handbook of all British Mosses," Unwin's "Dissections and Illustrations of British Mosses."

POLYCISTINA.—These animals are a family of Rhizopoda Radiolaria, the silicious skeletons of which form a series of very interesting objects for the microscope. They exist on every ocean floor and embedded in rocks, fossil Polycistina having been found at Oran, Bermuda, and Barbadoes, as well as in many other places. The silicious skeletons, or shells, as they are sometimes termed, with their prolongations, are aids in discriminating between Poly-

istina and Foraminifera, which former some of the species esemble. A section of Barbadoes rock, showing Polycistina *n situ*, is often a pretty, interesting, and instructive object. These objects may be obtained from Barbadoes earth, by oiling with its own weight of washing soda and a similar reight of water for one hour. After allowing to settle, the flock" must be poured off and the boiling with soda epeated, with intermediate washing several times. *Rhopaxanium ornatum*, *Podocyrtis Schomburghii*, and *Astromma Aristotelis* are, perhaps, the most beautiful of this class.

Books which may be consulted:—Haeckel's "Die Radiolarien," "The Micrographic Dictionary."

POLYZOA.—These interesting objects have, by the researches of modern inquirers, become much more known; hey are both marine and fresh-water, and may be found in nany rivers and canals.

On the sea-shore they are very plentiful, forming a crust n submerged rocks, or attached to stones, shells, and sea-reed. The individual animal is termed a *polypide*, and the olony a *cœnœcium*, which latter consists of an aggregation of ells or cups, often taking elegant and vegetable forms. Most Polyzoa are fixed organisms, but one at least—the *Aristatella mucedo*—is capable of locomotion.

One beautiful form, the *Lophopus crystallinus*, may be ound constantly in the Gorton Canal, near Manchester, and s much sought after by microscopists, visitors to the neighbourhood.

Of marine Polyzoa, the sea-mat, or *Flustra foliacea*, may e taken as a type; it is most plant-like and flexible. The ells are narrow at the base and rounded at the end, with cattered marginal spines; when rubbed betwixt the fingers t exhales an odour resembling oil of lemon. When collected, hey must be preserved in bottles or tubes with some of he water they were taken from, while it has been stated

that they may be preserved with expanded tentacles by dropping alcohol into the water; but this is not always successful.

Books which may be consulted:—W. Saville Kent's "Manual of the Infusoria," Johnstone's "British Zoophytes," "Micrographic Dictionary," Allman, "Freshwater Polyzoa" (Ray Society).

VEGETABLE PREPARATIONS.—The raw material for this branch of study may be found everywhere, the vegetable kingdom being able, perhaps, to supply more objects for the microscope than all the other classes together. Algæ, diatoms, ferns, fungi, lichens, and mosses, have already been mentioned, but the phanerogams alone are able to furnish matter for an immense amount of observation. The stems of trees and shrubs, the pine, fir, hornbeam, box, birch, beech, oak, chestnut, and elder, being easily accessible, are interesting when cut into sections. The leaves, seed, pollen, root-hairs, cuticles, raphides, and a host of other preparations, may be obtained from almost any plant, whether it be a choice specimen from the greenhouse or a common weed from the garden.

The starches form a study in themselves, and are specially interesting from many points of view; they are easily prepared, and should be kept dry, in small homœopathic tubes, for observation. The grains, such as wheat and barley, may be bruised and set aside for a few days to ferment, when, upon squeezing through a fine cotton cloth, the milky liquid contains the starch. Roots, rhizomes, and tubers require to be rasped, and the milky fluid strained in the same manner. After a time, the starch granules subside, when the supernatant fluid must be poured off and fresh water added, the whole stirred up, and allowed to settle again. After pouring off the water, the granules should be allowed to drain upon blotting-paper, and dried at the temperature of the air in a

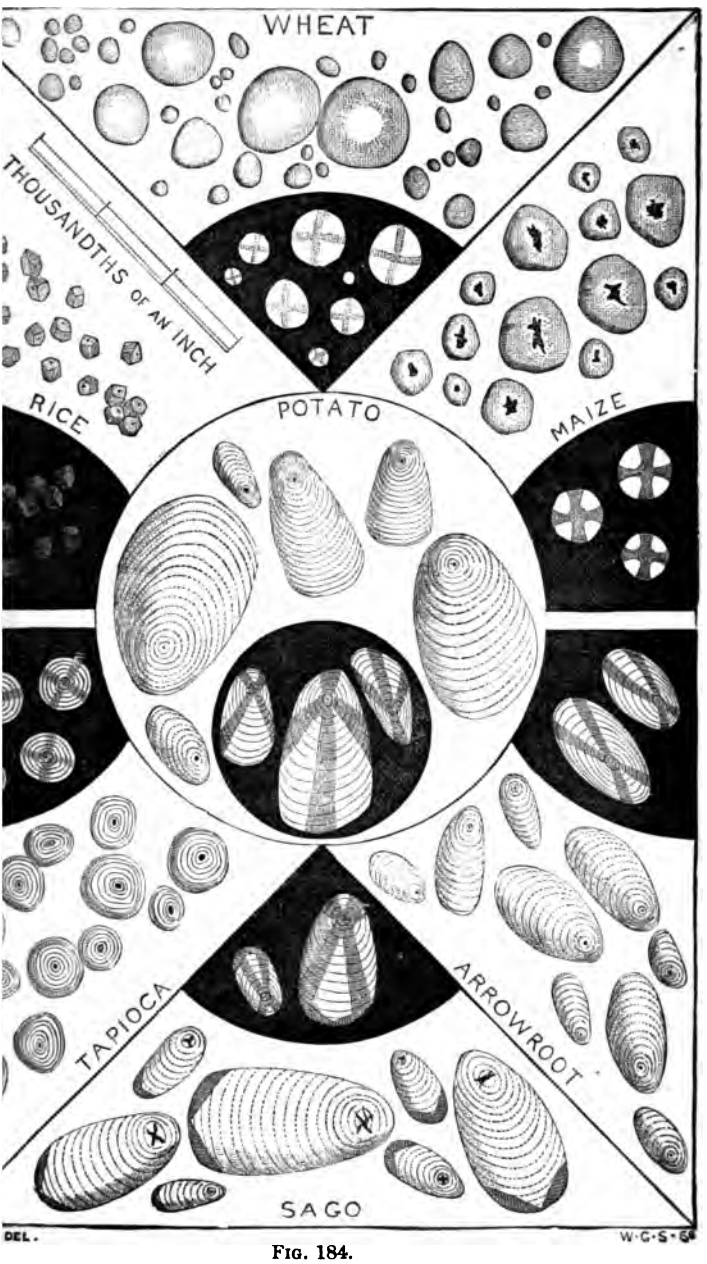

WHEAT
THOUSANDTHS OF AN INCH
RICE
POTATO
MAIZE
TAPIOCA
ARROWROOT
SAGO
DEL.
W·G·S·

Fig. 184.

warm room. Fig. 184 shows several of the commoner forms of starch.

Nothing special is required for the collection of raw material for vegetable preparations, without it be a tin case, for holding leaves, stems, or the whole plant, and squares of soft tissue paper, in which to carefully fold specimens. In collecting pollen, it is well to gather the complete flower, and

Fig. 185.

carefully isolate the specimens, in order that no confusion of the granules may take place. The pollen of a species of the mallow tribe is often used as a test object for low-power objectives. It is shown in Fig. 56.

Works which may be consulted:—Maôt and Decaisne's "Descriptive and Analytical Botany," by J. D. Hooker; Sachs' "Text-Book of Botany" (Clarendon Press); Balfour's "Manual of Botany."

Zoophytes.—This division includes the Hydrozoa and the

Actinozoa, both of which furnish many objects for study. In the first class we find the *Hydra viridis* and *H. fusca*, which may be collected, without much trouble, from streams and pools; it is visible to the unassisted eye, though it can only be satisfactorily examined by the aid of the microscope.

Fig. 185 shows *Hydra fusca* as it usually occurs, attached to *Anacharis alsinastrum*, and in the same manner it is often found on duckweed and other water plants.

Fig. 190.

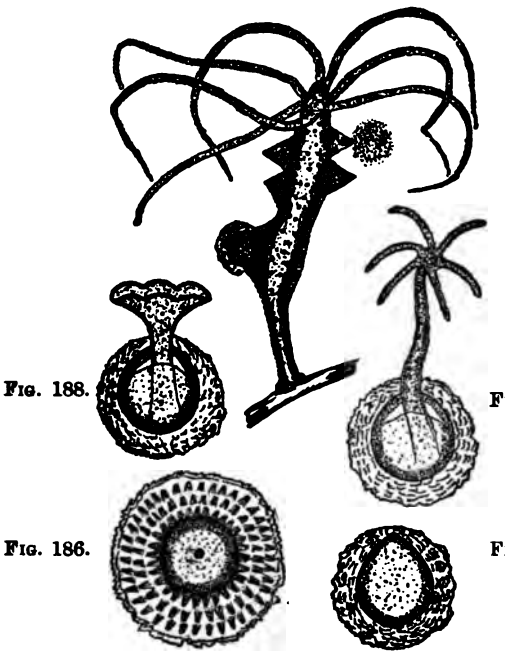


Fig. 188.

Fig. 189.

Fig. 186.

Fig. 187.

A very interesting paper on the development of the Hydra was published in vol. iii. of the "Microscopical News" by Mr. J. W. Dunkerley, who followed the development of the ova of *H. vulgaris*, and figures 186—190 will serve to illustrate his account of it. The ovum itself is shown in Fig. 186.

As the hatching approaches the envelope becomes thinner at one part, as in Fig. 187; a small opening then appears in the envelope, and the tentacles of the young Hydra protrude, as in Fig. 188. After some hours it becomes perfectly formed, and floats about, attached to the envelope for several days, as shown in Fig. 189, finally becoming a perfect Hydra. See Fig. 190.

Another order of the Hydroid Zoophytes is the Tubularida, of which *Tubularia ramea* is a specimen. It is commonly called the "branched pipe coralline," and is a marine organism; in fact, all the Tubularida are marine, with the single exception of the genus Cordylophora, which inhabits fresh water.

Amongst the Actinozoa are the sea-anemones, the coral polypes, and the Cydippe (*Pleurobrachia pileus*).

In the collection of marine objects, experience has taught the collector that the form of dredge by far the best for echinoderms is the tangle dredge. As many as nine specimens of the brittle star-fish *Ophiocoma rosula* have been taken at one haul, not one of which was injured in any way. By means of a pair of strong scissors, the specimens may be cut away from the tangles, and if they are intended for the cabinet, the fragments of hemp-fibre may be afterwards removed at the student's leisure by means of a pair of forceps. It is necessary to provide oneself with a duplicate set of tangles, as, after a large number of specimens have been taken, they become matted together, or so much may have been cut away as to render them useless.

This form of dredge is, however, of very little value when the larger forms of crustacea, molluscs, and polyzoa are desired, in which case it is better to have a small dredge made, the general plan of which will be found figured and described in Woodward's "Manual of the Mollusca."

The frame, or "scraper" as it is called, Fig. 191, is of

iron, is 12 inches long, $3\frac{3}{4}$ inches deep, and $1\frac{1}{2}$ inches broad. In front, and exactly in the middle of the two sides AA, a hole is drilled, into each of which a piece of strong iron wire, of the same length as the frame, is hooked, and the free end of each piece is bent in the form of a ring. To the two rings

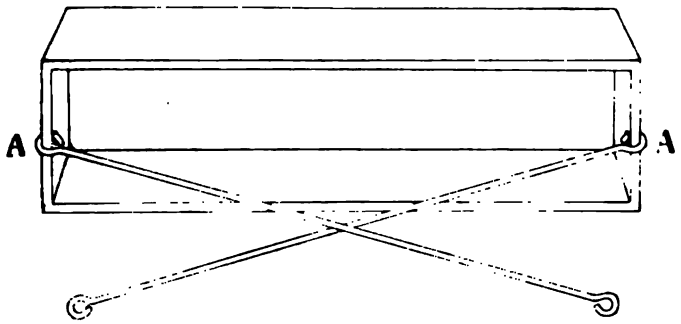


FIG. 191.

the towing line is tied. A writer in "Science Gossip" recommends that the line should be attached to one ring only, the two rings being tied together with a piece of spun

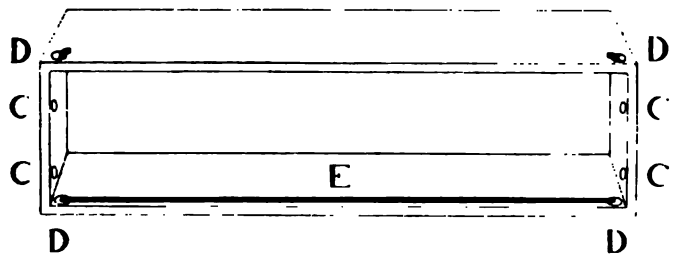


FIG. 192.

yarn: this arrangement being said to facilitate the liberation of the dredge when it gets fast to a stone. At the back of the scraper, Fig. 192, four holes are drilled on each side, those marked DD receiving the ends of the two lengths of strong brass wire, EE, which hold the net on to the frame, and into those marked CC, two V shaped pieces of brass wire, as

Fig. 193, are fastened, the object of which is to prevent the scraper from falling on its back, and so closing the mouth of the net. The net is an ordinary landing net of the best quality. It is important that the material of which the net is made should be very strong, on account of the wear and tear to which it is subjected; and, when new, the mouth should be considerably wider than the frame, to allow for the shrinkage which takes place after it has been in the water some time. It is also an advantage to have the width uniform throughout. The towing line is made of linen stay-cord, waterproofed. Though rather thin, it is quite strong enough for use with the dredges described. For a dredge of larger size, say 18 inches in width, unless used from a boat of large size, and in water over ten fathoms in depth, the collector should use the best and thickest linen blind-cord, waterproofed. The use of a thick rope is unnecessary for

Fig. 193.

small dredges. When the dredge is put into the water, the boat should be pulled rapidly for a minute or two; the line being allowed to run slowly through the hands until the dredge falls to the bottom; and as soon as the length of line run out is twice or three times the depth of the water dredged, the boat may be allowed to drift with the tide, or gently pulled if the tide is sluggish. The line should be held in the hands, and let go instantly, when a sudden strain indicates that the dredge has fouled. Sometimes a smart pull will liberate it; but it is generally necessary to retrace the ground. A good knowledge of the nature of the sea bottom

is very essential to successful dredging, and if it is necessary to engage a boatman, a preliminary trial trip should be made before finally engaging him, in order to test his knowledge and abilities. Boatmen who collect for public aquaria should be avoided, as a knowledge of the desiderata on their part too often results in the dredger being taken clear of all the productive grounds. A boat provided with a mast and sail, which can be taken down and stowed away during the dredging operations, is preferable to one provided with oars only.

CHAPTER X.

THE PRESERVATION AND EXAMINATION OF LIVING OBJECTS.

AFTER objects have been collected, they require to be preserved with very great care, or their future examination is likely to prove unsatisfactory. Most microscopic organisms are so delicate that they will not bear with impunity any great changes from their natural element, and though it is possible to send them to a distance by post, yet they should be well cared for on the instant of their arrival. It has been very common of late years to exchange living organisms with collectors at a distance, the specimens being sent in small glass tubes, which travel very well when properly packed and labelled. Directly such tubes are received by post, the cork should be taken out and the tube pushed through a suitable aperture in a flat bung, and then floated in water to preserve an equable temperature; in this way the organisms may be preserved until time is found for their examination.

It must be borne in mind that tube-dwelling Rotifers, Vorticellidæ, and many Polyzoa, if alarmed, retract their tentacles, and so, to a certain extent, are lost to view, and it is a well-known fact that many of the recipients of Mr. T. Bolton's tubes of living organisms failed to find the organisms

stated to be present because they had not given the tubes sufficient rest before examination.

The method of treating the spoils of a day's rambling must, of course, depend upon the nature of the objects culled; but if they are pieces of weed such as the *Anacharis alsinastrum*, they should be placed in a trough, such as is shown in Fig. 194, put into position at once on the stage of the microscope, and allowed a few hours to rest before the examination is made. The student should carefully examine the whole of the weed under the low powers in the trough, and it is very likely he will be repaid by seeing some younger individuals just commencing the building of their tube, and he may possibly find others, in a still earlier state, swimming or creeping amongst the leaves. The trough shown in Fig. 194 is not always the most convenient for a good all-round examination of an object. It is made of a thick glass base plate and ends, while two pieces of thinner material form the back and front, which is generally of the same degree of thinness as the thinnest slips are cut from. In size this trough should measure $\frac{6}{10}$ of an inch in width, 2 inches in length, $1\frac{1}{2}$ inches deep at the front, and 2 inches at the back, the base plate and ends being made of glass $\frac{1}{5}$ of an inch in thickness and cemented together by marine glue.

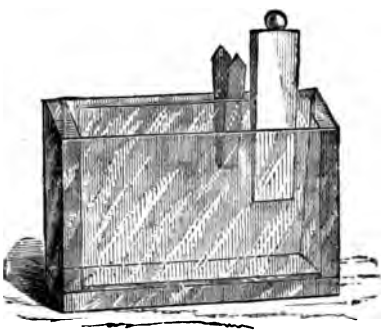


Fig. 194.

For higher powers, the form of trough shown in Fig. 195 is desirable. It consists of a glass slide, 3 by $1\frac{1}{2}$ inches, upon which is cemented with marine glue an ebonite or glass semi-rectangular piece, as shown in the figure; the half of a flat

indiarubber ring will answer admirably, the front being formed of a piece of thin glass.

In use, nearly fill the trough with water, place it on the stage of the microscope, and incline the body of the instrument so that the observations may be made with comfort; adjust the lamp and concave mirror so that the most intense and central light is thrown through the instrument; and lastly, adjust the diaphragm until most of the marginal rays are cut off, leaving only sufficient light to see the object clearly.

FIG. 195.

With many objects, say such as may be attached to a leaf of Anacharis or a piece of duckweed, all that is necessary is to place them on a glass slip, to add a drop of the requisite medium, and to cover with a thin cover glass. The ordinary slip, with a ledge of glass cemented to its lower edge, as shown in Fig. 196, is very useful, as it saves the stage of the microscope from corrosion when marine organisms are being examined.

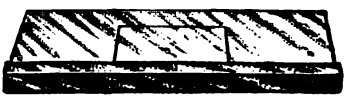


FIG. 196.

The trough illustrated at Fig. 194 is adjustable. The object can be pressed up to the front plate by means of the spring and wedge shown in the illustration.

Another adjustable form of trough is Botterill's, which consists of two brass or ebonite plates bolted together, as shown in Fig. 197, the plates of glass being separated, according to the space required, by an ordinary indiarubber ring of the requisite thickness. The trough can thus be taken apart and the glasses cleaned, or a broken front replaced without the

trouble of cementing, the glass sides being sufficiently thin to allow the use of high power objectives.

A microscopic life-slide, also devised by Mr. Botterill, is shown at Fig. 198; the advantages claimed for it are: the facility with which it can be used and cleaned; its reversibility, allowing either side of the object to be examined

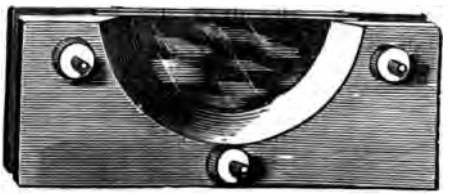


Fig. 197.

through thin glass; the provision for renewing the supply of water without disturbing any part of the apparatus, thus enabling objects to be kept under examination for an indefinite period; the same arrangement also allowing of the introduction of colouring matters, as carmine and indigo; and lastly, its moderate cost and durability.

For Confervæ, small Infusoria, and similar organisms it is sufficient to place the object on the bottom glass, with a drop

Fig. 198.

of water, and apply the covering glass in the same manner as when using a glass stage-plate. When a thicker layer of water is required, a narrow ring of vulcanite, cork, or other suitable material, of the requisite thickness, should be placed

on the lower glass, and the object put in position, the covering glass being finally applied as in mounting objects in a cell. The supply of water can be maintained by putting a drop occasionally in one of the side "wells," keeping the slide, when not under examination, in a small damp chamber, to prevent evaporation. To change the water, supply through one "well," and draw out through the other by means of a roll of blotting paper.

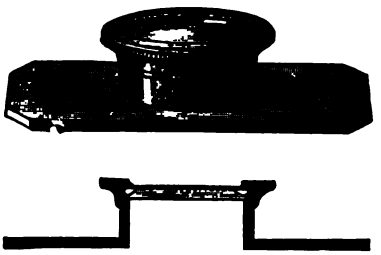


FIG. 199.

Messrs. Thompson and Capper, of Liverpool, were the original makers of this slide, and also of Botterill's zoophyte trough, illustrated at Fig. 197.

The simplest and least expensive method of examining

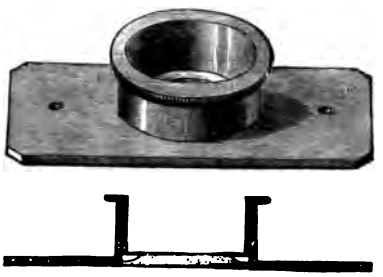


FIG. 200.

the Infusoria, and other moving micro-organisms, is by the use of the "Rotifer trap," of Mr. F. Bedwell. This consists of a few filaments of cotton wool placed upon the under glass of a live-box (Figs. 199, 200). The organism contained in a drop

of water is then run over it, and eventually becoming entangled amongst the fibres, is kept comparatively still, by which its form can clearly be made out.

Two forms of cages or live-boxes are shown in Figs. 199 and 200. They are, however, not all that can be desired. Just so much pressure must be applied to the cap as is necessary to keep the animal still, and no more, or it will be crushed and distorted, so that the cap requires much dexterous manipulation. Oftentimes such objects as Entomostraca, &c., will not display themselves to advantage, so that the cap requires loosening, to be again squeezed down at an opportune moment. Moreover, it is generally found that some particular organism will get near the periphery of the cover, and in this

Fig. 201.

all the interest may be concentrated, yet the objective will not reach it if it happen to be of the form of Fig. 200, and if we adopt the form of Fig. 199 we are often precluded from using the achromatic condenser to any portion but the centre of the slide. Instead of this we may use the compressorium as made by Messrs. Beck, Ross, Collins, and others, for use with high powers; but Piper's form, made by Mr. Swift, and illustrated in Fig. 201, will be found more convenient.

The most delicate pressure can be applied by means of these instruments, and all such intended for real use should be reversible, so that the objects may be easily viewed from both sides, and this can be done with the form shown in Fig. 201.

A form of compressor, differing from most others, has been devised by Mr. Holman, in the States. The top, or mica cover, is fixed, while the lower and thicker plate of glass is raised or depressed by means of a screw nut and spiral spring. The employment of a thin mica cover is certainly an improvement, and one which English opticians would do well to copy. In this we imagine every practical microscopist will concur, as the breakage of a glass cover in the middle of an interesting observation is, to say the least, vexing.

The directions already given are applicable to all micro-organisms attached to weeds. Large active organisms, such as larvæ, annelids, entomostraca, too large to be readily picked up by a dipping tube, may be removed on the point of a

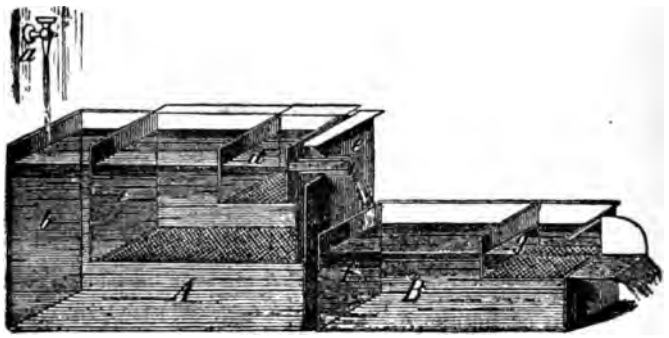


FIG. 202.

small sable pencil. A drop of water should previously have been placed on the centre of the live-box or compressor, just sufficient in quantity to allow the animal room to move about naturally; then, just touch the drop of water with the point of the sable pencil, with which you have picked it up, and it will most likely free itself, or else it must be carefully pushed off the brush by a needle mounted in a short wooden handle.

A very good study is the hatching of fish-eggs, and watching their development until the absorption of the

umbilical sac; for this purpose Max Borne's incubator, as shown in use at the Fisheries' Exhibition of London, in 1883, is of great use, as it may be constructed on a small scale by the microscopist himself. It is illustrated by Fig. 202.

The eggs are placed on the wire-netting bottom of the tray *c*, and the water entering the outer division *b* of the hatching trough, flows upwards through the hatching trough and ova, and is discharged at the spout *e* of the apparatus. Immediately under this spout, which is at first quite open and unguarded, to allow the empty egg-shells to be carried off by the current, a catch-trough, B, is placed, in order to catch such fish as may have found their way out of the incubator. No preparations of any kind are needed to put

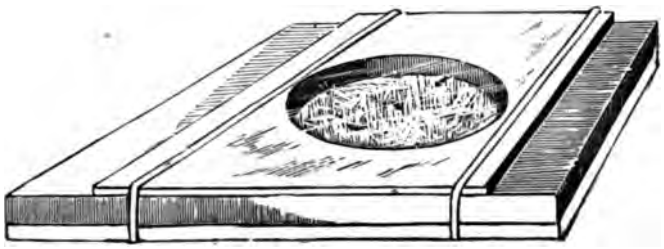


FIG. 203.

the apparatus in working order; all that is required to set it in operation is to furnish a constant supply of water, at the rate of about half a gallon per minute.

So far, aquatic organisms only have been dealt with; it is true that on page 226 the construction of a vivarium has been described, but for keeping and breeding smaller insects, as *Podurœ*, a much simpler apparatus will suffice. Fig. 203 shows the construction of what may be termed a Podura cage, devised and illustrated in the "Monthly Microscopical Journal," by Mr. McIntire. The glass plate, with a large perforation

in the centre, is covered on each side with a thinner glass, and held in position by two india-rubber bands. This is a very useful cage for many purposes, and no doubt many other and similar contrivances will suggest themselves to the earnest microscopist when he has work of any particular kind to do.

CHAPTER XI.

MICRO-DISSECTIONS.

MANY of the objects mentioned in the preceding chapters cannot be examined under the microscope without subdivision or previous preparation, either on account of their size, or because the parts lie in different planes, the transparent portions being hidden from view by others of an opaque character.

This is so with most insect life, especially the larger species, though, on the other hand, the more minute kinds, such as the *Acarus domesticus*, or cheese-mite, are generally so transparent as to require no special treatment.

Beetles and other insects, flies, snails, frogs, and newts are very good examples of the former class, and the student will do well to practise the dissection of these objects. He will find it absolutely necessary to acquire a good knowledge of dissection, in order to gain a correct insight into the relative structure of the insect economy.

We can hardly describe in words how to proceed in each individual case. Experience will come by practice, and the student will find that each subject becomes more and more easy, especially if, before commencing in haste with the needles and scissors, he will study the general arrangement of organs in his subject, by reference to some one or other of

the many standard works in existence, obtainable at any free library.

One remark, however, is necessary. Do not hurry on with imperfect dissections of a host of subjects; stick to one insect until a good knowledge of its anatomy has been gained, and by no means take the trouble to prepare and mount any imperfect specimens. Persevere until the dissections are perfect, and then spare no trouble or pains in preserving them for future reference.

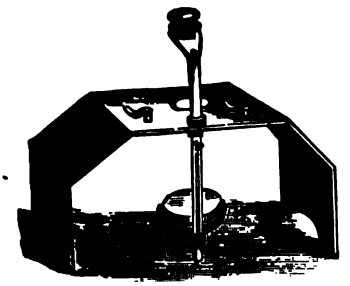


FIG. 204.

Dissections may be carried on with but few instruments; a few small-sized troughs, knives, scissors, needles, and camel-hair pencils are nearly all the requisites. The dissecting microscope must be left to the taste of the operator, who will find many forms to select from.

Fig. 204 shows Collins' cheap dissecting microscope, of which a large number are in use in Professor Henslow's botanical classes; while the next figure (205) is an illustration

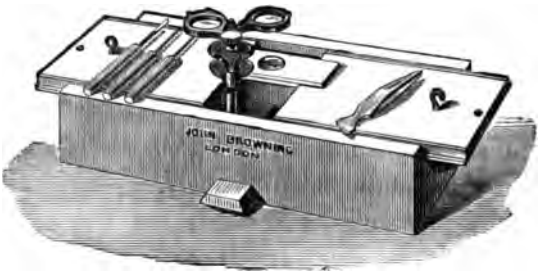


FIG. 205.

of Houston's botanical dissecting microscope, which may suit botanists who wish for nothing more than a steady stand for a pocket magnifier.

This ingenious little instrument is intended to provide working botanical students with a convenient and serviceable dissecting microscope at a moderate cost. The box measures, when closed, 9 inches long, 4 inches wide, and 2 inches deep, and is so constructed that, by using a divided sliding lid (acting as a support for the dissecting stage), a rest for the wrists is secured while the hands are employed in dissecting.

The duplex lens, giving three powers, 4, 6, and 10 diameters, is screwed to the end of a brass focussing tube moving upon a brass pillar attached to a sliding bar at the

FIG. 206.

bottom of the box. The lens may at any time be unscrewed and carried in the pocket.

The dissecting stage is a cork slide, plain on one side for general work, but provided with a shallow cell on the other, for the dissection of such objects as small glossy seeds, which "fly" under the needles. A pitted glass slide, to be used when the object is best dissected under water, is also provided. A cutting needle, two dissecting needles, and a pair of small forceps are also included, and the whole is sold at the moderate price of 6s. 6d.

Fig. 206 is a representation of Lawson's dissecting microscope, as made by Mr. Collins—a very handy and compact instrument, containing, as it does, in the unfolded case the knives, needles, and scissors for the operator's immediate use.

Fig. 207

Zeiss makes a large dissecting microscope of special pattern, shown by Fig. 207, which is exceedingly handy to work with. It consists of a heavy square stand and a large stage, to which is attached leather-covered hand-rests.

There is a large concave mirror underneath the stage, and the adjustment of the lens is made by means of a rack and pinion. In all Zeiss's dissecting stands ample provision is made for resting the hands, a necessity quite overlooked in many English instruments.

If the erection of the image be desired, it can be obtained by the use of the draw-tube and erector already illustrated by Fig. 69, and this method has many advantages over the use of the Stephenson binocular. The erector screws into the end of the draw-tube, and using the A eye-piece with a ½-inch objective any amplification from 2·5 to 60 diameters may be obtained, or from 12 to 260 diameters with the D ocular. It must not be forgotten, however, that the use of the erector disturbs the corrections of the objective, producing spherical and chromatic aberrations. This is so great that, when using the A eye-piece, and a ½-inch objective, an object may be brought to an indifferent focus at any distance between 1 inch and 2½ inches from the front lens upon the instrument shown in Fig. 19; but at 1½ inches perhaps the sharpest image is formed. The way to remedy the effect of these aberrations is to use the C or D eye-pieces, when the object runs in and out of focus very sharply, and as the peripheral rays are not gathered in, there is but little aberration of either kind visible.

Position of Draw-tube.	A Eye-piece.			D Eye-piece.		
	Distance of Front Lens from Object.	Size of Object in Field.	Amplification in Diameters.	Distance of Front Lens from Object.	Size of Object in Field.	Amplification in Diameters.
	in.	in.		in.	in.	
Full home	1·30	0·75	2·5	1·05	0·40	12·0
Withdrawn ½ inch	0·60	0·40	7·0	0·44	0·15	30·0
,, ½ ,,	0·36	0·20	11·0	0·36	0·09	50·0
,, 1 ,,	0·23	0·12	20·0	0·23	0·05	90·0
,, 2 ,,	0·17	0·06	40·0	0·17	0·03	180·0
,, 3 ,,	0·15	0·04	60·0	0·15	0·02	260·0

The preceding table will show the use of the erector and

draw-tube, used upon the stand in Fig. 19 with a ½-inch objective of 50° air angle.

For cheapness, there is nothing better than the watchmaker's or engraver's eye-glasses. These may be fixed into any form of stand the ingenuity of the student may devise, and with these alone very much work may be done, quite as much, perhaps, as he will be able to execute for some time.

The subjects for dissection are usually operated upon under water or some other fluid, in dilute alcohol, or even in glycerine. This is a very convenient medium in which to carry on minute dissections, as the operation can be performed with much more certainty than is the case in more mobile fluids.

In whatever fluid the specimen for dissection be immersed, a trough is necessary to hold it, such as shown in Fig. 208, and this may be either of gutta-percha or glass,

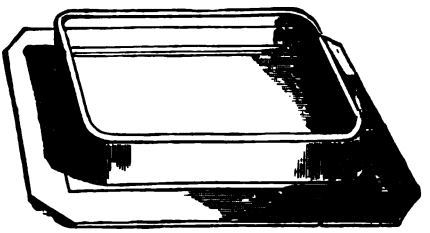


Fig. 208.

not too large, but roomy enough for working, and also for the disposal of loose matter. Half an inch in depth is ample (except for special subjects), as, if deeper, the sides are apt to interfere with the free use of the needles and fingers.

If the trough is of glass, a suitable bedding may be run in to a depth of a quarter of an inch, to which the subject can be pinned down. A translucent bottom may be made of a mixture of napthalin and stearine, an opaque bedding of equal parts of resin and beeswax, thinned to the necessary

istency with unsalted lard, and coloured black with
-black, or white with oxide of zinc or dried china-clay.
While dissecting, no instruments are more handy than
nary sewing needles, thrust with the eye first into cedar-
d handles. They may be bent to any required shape
e heated in the gas or a spirit-lamp flame, and after-
ls rehardened by heating and plunging, while hot, into
They must be kept well polished, as if any roughness

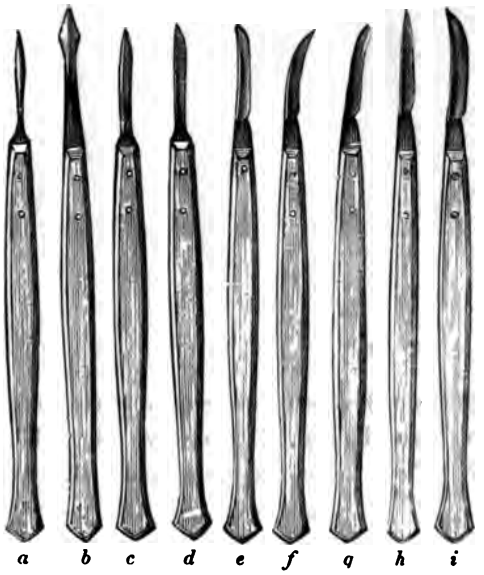


FIG. 209.

rail on their surface it is next to impossible to produce
thing but torn and disfigured dissections.

Camel-hair pencils are indispensable agents for the
oval of loose matter, torn and unrequired portions of
insect anatomy, and also for the arrangement of the
ected portions.

The knives used in dissecting should be of good steel,
kept in order by the occasional application of a Washita
stone. Some of the most useful forms are shown in

Fig. 209—two or three will, perhaps, be selected; the author prefers the forms shown at *a*, *b*, *d*, *f*, and *h*. Nearly every microscopist prefers to work his own way, and with his own choice of instruments. The spear-head needles sold by Mr. Ward, of Manchester, will be found exceedingly useful.

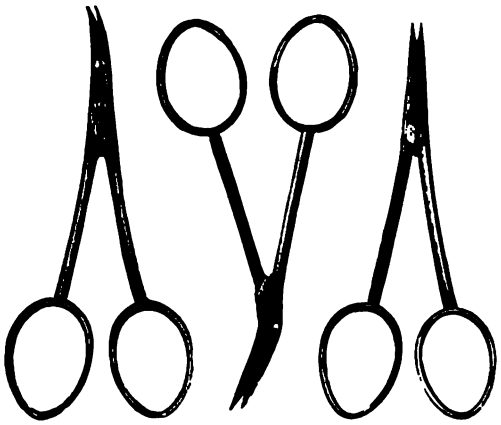


Fig. 210. Fig. 211. Fig. 212.

Three kinds of scissors are generally required by the dissector, the curved, Fig. 210, elbow, Fig. 211, and plain, Fig. 212. They must be well made, and kept carefully

Fig. 213.

cleaned and free from rust. The application of mercury ointment will usually go far in protecting steel instruments from this enemy.

Another pair of scissors is also much used in minute dissections, one leg of which is fixed in a light ebonite handle, the blades being kept apart by means of a spring.

The pressure of the finger on the loose leg causes the blades to close, and it will be found after a little practice that the instrument may have many applications. It is shown in Fig. 213.

The steel forceps, shown in Fig. 214, are necessary

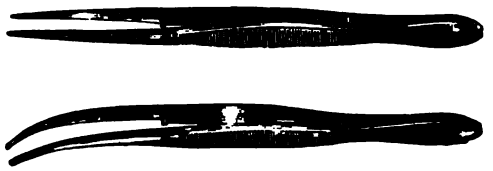


Fig. 214.

adjuncts to the dissector, and he should be careful to select those of but medium strength in the spring, and such as can be depended upon for seizing an object finely yet firmly, and not at all liable to twist aside and spoil the subject under dissection. A pair of curved forceps, as well as a pair of the straight variety, with fine points, are necessary.

A bull's-eye condenser for concentrating light upon the object, and a small glass syringe for washing subjects and removing or adding to the liquid contents of the trough, complete the dissector's outfit.

And now, having his apparatus ready, the student will naturally be looking about for something to dissect. He need not seek long at any season of the year, but in the event of his not being able to fix on what he shall commence with, the blow-fly, *Musca vomitoria*, is suggested.

Plenty of work will be found in the blow-fly if it be done properly, and after this the student may take in hand the water-beetle, *Dytiscus marginalis;* the grasshopper, *Locusta viridissima;* or the common house cricket, *Acheta domestica.*

In the above, the student will find the tongues, antennæ, eyes, wings, legs, segments, spiracles, and tracheæ, an

interesting study, and besides these organs, gizzards will be found in the last three.

The insect (if not too large) should first be held in the stage forceps as shown in Fig. 108, or in any other convenient manner, and examined with a low power (say a 2-inch objective), to show its general character, and give an insight into the manner in which the appendages should be dealt with.

The proboscis of the fly may be obtained by pressing the thorax, so as to cause protrusion, placing the organ upon a strip of glass, covering with a thin square cover, and severing with a sharp knife. If the insect has been killed with chloroform, the organ will generally be found protruding. Just a word, *en passant*, to encourage the student to display his dissections naturally, in order to give observers an idea of the real use of the various organs in the insect economy. This cannot be better illustrated than by reference to this proboscis, one of the late Mr. Topping's favourite preparations. The more natural condition should be mounted in glycerine, and though not so pretty an object, is at least truthful.

Put no insect to pain. Kill it at as early a stage in the enquiry as is possible, either with chloroform, bruised laurel leaves, or by means of the cyanide of potassium bottle now so often used.

Proceeding with the blow-fly, the wings may be detached from the thorax by means of the knife, scissors, or forceps, the legs taken off at the thigh, the halteres or poisers detached, and the antennæ cut from the head, completing the list of appendages. The carcase must now be pinned down in the trough, or fixed to the bedding by warming a spot with a hot iron, fixing the subject into the melted stratum, and the integument carefully slit up with a fine pair of scissors upon both sides. The chitinous skeleton must then

be raised with the forceps, and the attachments cleared away with the aid of a blunt needle and a spear-headed instrument, when, if tolerably well performed, the whole of the organs will be seen *in situ*. The subject should now be left in a mixture of glycerine and water (equal parts of each), for about twelve hours, after which treatment the organs may be

FIG. 215.

FIG. 216.

readily dissected. Dilute alcohol is very useful when dissecting the nervous system of insects. A more or less prolonged immersion hardens the nerve fibre, which the student will find requires very delicate handling. It is not difficult to find

and take away the tracheal system (though the beginner will find this easier to prepare from the silk-worm or caterpillar), and the muscles which are used for raising and depressing the wings during flight may be found without much trouble.

The digestive apparatus varies somewhat in different insects, and as an aid to the student, the arrangement of these organs is given as they appear *in situ* in the water-beetle (Fig. 215) and the blow-fly (Fig. 216).

The fly lives by suction, and, therefore, the food does not require any unusual amount of mastication. It therefore has no gizzard. The water-beetle, on the other hand, has a gizzard placed at the end of the œsophagus, or gullet, its object being to more completely triturate the food before it passes into the stomach. The gizzard is composed of hard muscular tissue, and nearly always covered internally with hard growths called teeth.

In Fig. 215, A is the œsophagus or gullet, B the gizzard, D the stomach, E the small intestine, while the large oval-shaped sac at the extreme left is the large intestine.

If the gizzard only is required, the best plan is (after killing) to hold the insect under water, and with the forceps to draw the head from the body. Most of the internal organs will by this means be drawn out, when the gizzard can be detached with the scissors from its place below the gullet, washed, and opened out with knife and needle.

After having proceeded so far, the student will do well to examine the nervous system, and, last of all, when the inside has been cleared out, the segments and spiracles may follow in their turn.

Insects intended for dissection should never be allowed to become dry, and if they cannot be treated at once, they should be preserved in dilute glycerine, dilute acetic acid, or weak spirit, according to what is required of them afterwards.

The student, perhaps, may not have patience at first to go through the whole set of dissections from one insect, and, therefore, such objects as the saws of the different saw-flies may be attractive.

The wasp and the bee may also be pressed into the service of the dissector, and between these two he will find some remarkable differences: in the wasp, the poison-bag is separated from the body of the sting by a long tube, and the bag itself is so well covered with muscular fibres that it is impossible to separate them from it without tearing the whole to pieces.

The object of dissecting, after all, is not to be able to prepare pretty slides merely, but to gain a knowledge of the anatomy of our subject, so that we may understand more thoroughly the various transformations which take place. It may not be possible always to isolate each organ we wish, and in that case a careful study should be made of the organs *in situ* sometimes by polarised light, and what we have actually seen may be transferred to paper by any of the methods described in the chapter on "The Delineation of Objects."

Another study, having an interest with many, is that of the tongues, palates, lingual ribbons, or odontophores, of molluscs.

The subjects for dissection should be killed by dropping them into glycerine, and so preserved until they are required. The dissection should be carried on under water, in the usual way, though the mollusc needs but little pinning down.

Let the student start with the common periwinkle, *Littorina littoralis*, or, perhaps, better still, with *Patella vulgata*, or common limpet. In the former, the lingual ribbon will be found coiled up like a watch-spring by opening the back of the animal, and this place will be found generally the best of all to examine first.

In *Patella vulgata* it is only necessary to remove the foot,

or broad flat disc forming the lower surface of the body, when the lingual ribbon is exposed to view, and appears as shown in Fig. 217.

The *Trochus zizyphinus*, or pearly-top, may also form a subject, as it is found in tolerable profusion on our shores; and the whelk, *Buccinum undatum*, will also furnish new specimens.

In *Trochus zizyphinus* the lower lip of the mouth must be exposed

Fig. 217.

from above, when the lingual ribbon will be found lying upon it. In the whelk, the trunk contains the whole of the ribbon, and may be seen by opening the back just behind the tentacles.

In Fig. 218 the periwinkle is well displayed: *f* is the foot, *m* the muscle for withdrawing the animal into his shell, *g* the spittle glands, *th* the throat, *s* the stomach, *r* the odontophore, *br* the breathing gills, *a* anus, and *o* the ovary carrying eggs.

The next illustration shows an oyster lying in its shell (Fig. 219); *m* is the lower half of the mantle, *m'* a piece of upper half, *g* the breathing gills, *h* the heart, *lv* the liver, *lp* the lips, *o* the opening of the mouth, *a* the anus, *ms* muscle holding shells together, and *c* elastic cushion keeping shells apart.

Dissection of vegetable matter is usually preceded by prolonged immersion in water, in order to soften the parts to be separated; but very often indeed the tissues withstand this action, and so recourse is had to various other solutions which are found necessary. Caustic soda in dilute solution is a favourite re-agent for the differentiation of vegetable matter. It is the solution used by the

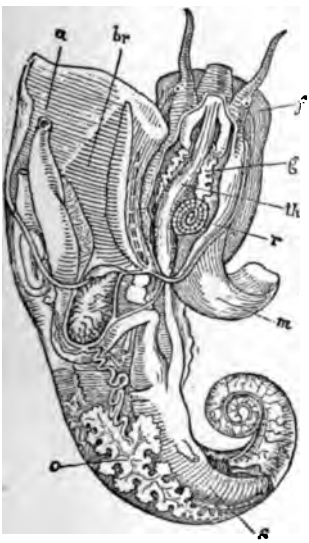


FIG. 218.

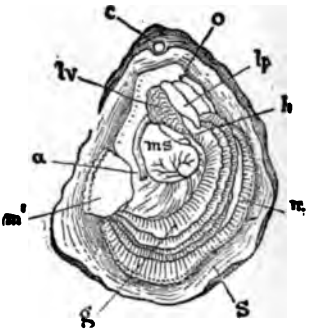


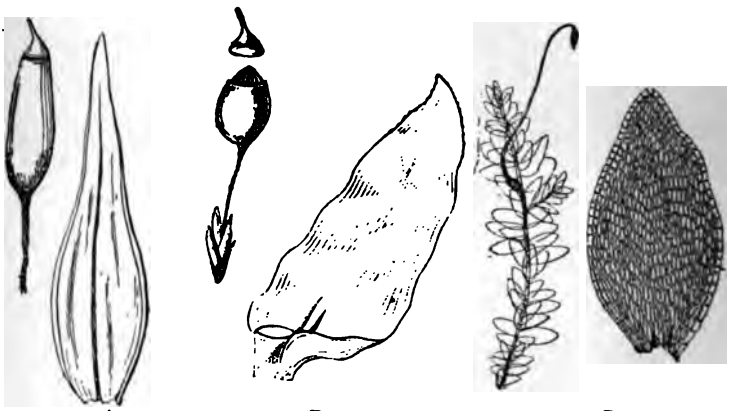
FIG. 219.

paper-maker for the disintegration of the leaf of esparto grass, the fibres of which furnish him with a valuable paper-making material.

Fibro-cellular tissue may be conveniently studied in the leaves of orchids, where it is easily found, underneath the cuticle, after maceration. Woody tissue, often the chief component of certain plants used in the arts, is composed of

fibro-vascular vessels aggregated in bundles, as in flax for instance; and the same may be said of jute, china-grass, and many other products. These fibres may be examined by macerating in water, during which a fermentation sets in and the fibrous bundles consequently split up; the same effect may be produced by boiling with dilute caustic soda solution.

Spiral fibres also form interesting objects for study. They occur sometimes in cells alone, and at others in conjunction with what have been styled bordered pores, as in the yew and araucaria, while the pores are found alone in the pine. Most woods, when in thin shavings, are easily decomposed by boiling with dilute nitric acid, when the fibres can be pulled



A B C

FIG. 220.

asunder, or by boiling with caustic soda solution under pressure.

Spiral vessels are even more interesting than any of the foregoing, and easily procured. From the stem of the leek they are separated with ease, while from the petiole of the common garden rhubarb they can be obtained in plenty by searching in a pot of this preserve.

Perhaps of all exercises the complete dissection of a moss is the best for botanical students; the various parts mounted on an ordinary slip under a three-quarter thin glass cover are at once a type slide and an object of beauty. A glance at Fig. 220 will show this, a representation of *Homalothecium sericeum*, the silky Feather Moss, is shown at A; *Neckera crispa* at B, and *Hookeria lucens* at C.

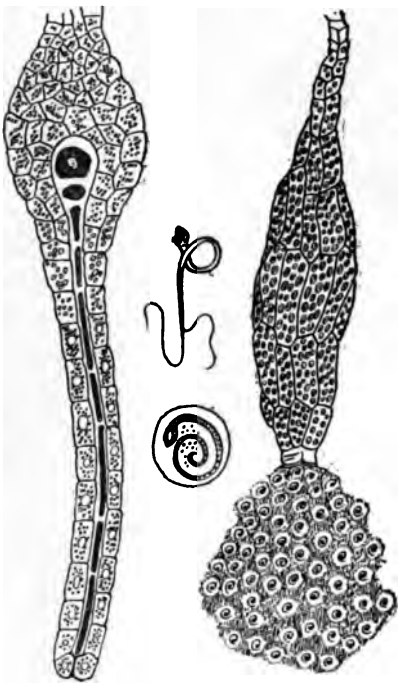


FIG. 221.

The antheridia, or male organs of the mosses form a very important study; they are of an elongated club shape, and when ripe, assume a red or yellow colour. They are shown in detail by Fig. 221. It will be seen they are built up of cellular sacs, containing chlorophyll granules; when ripe, a slit opens across the apex, through which the antherozoids,

still enclosed in their mother cells, are discharged as a thick jelly. When the gelatinous matter has been dissolved by water, the antherozoids escape and swim about in the free state.

Scalariform vessels are to be met with in the roots of ferns. The *Pteris aquilina* perhaps displays them as well as any other plant; but it is a moot point whether they are better seen by the examination of a dissected portion, rather than a section made in a diagonal direction. It is true that much can be made out from sections of the various parts under study; but without dissection we should be very apt to arrive at erroneous conclusions concerning the form and situation of parts, our views often being modified by the light thrown upon our subject by its employment.

Structural botany, or organography, can scarcely be studied to advantage without the student becomes an adept at vegetable dissection; and the same may be said of physiological botany, or the study of the functions of living plants. There is much to be learned yet respecting the manner in which inorganic materials are transformed into things which live; and anyone who has attempted to work in this most intricate branch of physiological study will be ready to acknowledge how pleasant such work becomes if the art of vegetable dissection has been previously mastered.

The growth of a stem or branch of any exogenous shrub from its bud, if faithfully carried out, may become a pleasurable pastime for many months, especially if the dissections which such a study entails be supplemented by the operations described in the next chapter

CHAPTER XII.

SECTION-CUTTING.

IN the preceding chapter the necessity of dissection has been shown, in order to gain an insight into the economy of things. It is none the less important, however, to be able to cut a thin slice of any given object, in order to show the details *in situ*.

We should be very careful not to form hasty opinions, from the observations of sections alone, as vessels will appear of different shapes according to the plane in which they have been cut. Circular vessels cut through obliquely give an oval outline; but in the direction of their length, squares.

Section-cutting may conveniently be divided into three methods:—

1. Without the use of the microtome (section-cutter), and even before hardening or otherwise preparing the subject.

2. With the microtome, such subjects as the stems of plants, leaves, animal preparations, and the like.

3. The cutting of hard substances, coal, rock, bone, and subjects of this nature.

To proceed with the first method, we find some workers cutting slices on or between pieces of cork, without the use of

any appliance whatever, save a knife or razor. Others use even more primitive methods, and perhaps, by long continued practice, may arrive at fair results, though the author has never seen uniformly thin and good specimens cut by the hand alone.

Some when examining animal tissues "prefer to snip off a piece with a pair of sharp scissors," or "cut a piece off with

Fig. 222.

a sharp scalpel," but all slides prepared in this manner are mere fragments compared with the splendid sections of Marsh, Cole, Wheeler, Norman, Ward, and others.

Fresh animal tissues, such as kidney or liver, are frequently cut with Valentin's knife (Fig. 222).

The instrument is formed of two narrow blades lying parallel to each other, their distance being regulated by means of a fine screw. The knife is used immersed in

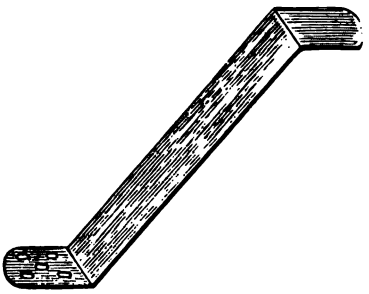


Fig. 223.

water; it is drawn through the tissue to be cut in a saw-like fashion, the sections being afterwards disengaged by shaking the blades gently in water. Dr. Maddox's form of this knife is one with triple blades, so that a double section might be cut, to show opposite but contiguous surfaces.

Dr. Sylvester Marsh, who has written a small manualette upon the subject of section-cutting, has devised a simple but useful spoon for lifting thin sections. It is shown in Fig 223.

After all, these methods of cutting are exceedingly limited in their application. They may be necessary at times, when it is desired to make a hurried examination; but the *student* is certainly advised never to make hurried examinations.

We now come to the second division, where sections are cut in the microtome, of which there are several forms, constructed in accordance with the requirements of the originators.

The most common form of section-cutter is shown in Fig. 224. It is generally used for cutting sections of wood,

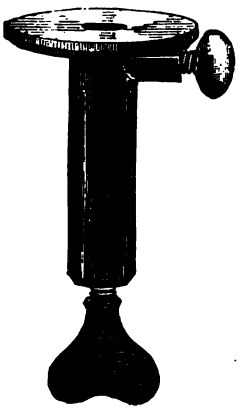


FIG. 224.

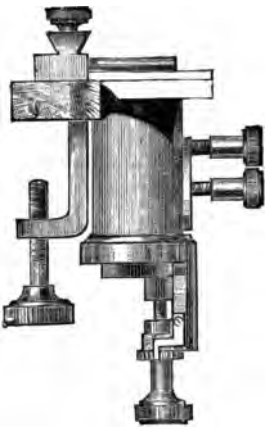


FIG. 225.

stems, and other semi-hard substances. The substance to be cut is kept firmly in position by means of the side screw, and the quantity necessary for each section sent up by the screw below.

Another section-cutting machine, used for the same

purpose as the preceding, has been devised by Mr. Hailes, and shown in Fig. 225. It is sold by Messrs. Baker, of Holborn. All the faults of the old form have been remedied, and for cutting semi-hard substances it is the best of its kind.

For cutting soft tissues and animal preparations generally, there are few instruments so handy as Cathcart's freezing microtome, shown in Fig. 226, and certainly none so cheap in price. It consists of the framework and the

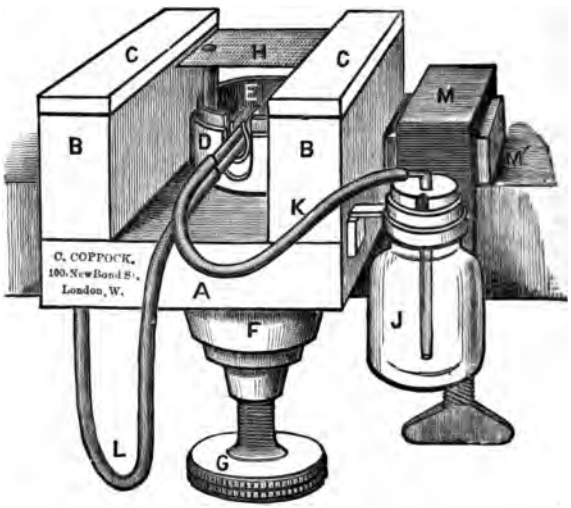


FIG. 226.

mechanism for raising the section. The framework is of ½-inch mahogany, and is in the form of a base, with two upright parallel pieces screwed on to it. The base (A), which is about 2½ by 4 inches, is bored to allow the tubes for raising the section to pass up between the parallel pieces, and has a projecting part at one side to allow of its being clamped to the table (M). The two parallel parts (BB), which are of the same ½-inch mahogany, stand about 1¼ inch apart; they are 4 inches long, and, rising to 1 inch high, each carries on the upper surface a piece of 1¼-inch plate glass (CC), of the

same length and breadth as itself. This is to support and steady the knife as it is pushed across the tissue to be cut, while the fact of the tissue coming up between the plates allows that part of the knife which is to cut the specimen to be kept free of contact until it touches the tissue.

The method of raising the section plate is as follows :— About 2 inches of accurately-fitting double brass tubing are taken, and into the outer one (D) the nut (F) of a fine screw is firmly soldered at what is to be its lower end. The inner tube (E) has the section plate fixed to its upper end by two screws, with, however, two small pieces of vulcanite intervening between the plate and the tube, so as to disconnect them as much as possible, and into the lower end of the inner tube a transverse bar is fitted, against which the screw coming through the outer tube presses when it is desired to raise the section plate to which the inner tube is attached. By means of a small screw-nail fixing the outer screw to the bar in question, the inner can be withdrawn, as well as pushed up whenever that movement is required. A milled head (G) has been substituted for the ordinary capstan arms for turning the main screw round.

The spray points are introduced at the requisite distance below the section plate by cutting a narrow slot through both tubes, and fixing to the inner one a piece of bent brass, into which the spray points can be pushed and held firmly, while a small shoulder on the latter prevents them from passing beyond the centre of the under surface of the plate.

Finally, the ether bottle (J) is fastened to the side of one of the upright pieces of the framework by a simple hook and eye, the hook being fixed to a collar round the neck of the ether bottle, and the eye to the side of the framework in question. It will be seen from this description that, with the exception of the fine screw for raising the tissue, the details of the mechanism are very simple, hence the low price at

which it can be sold, and in practice it has been found to work admirably. Mr. Cathcart gives the following directions for its use:—

1. Place a few drops of mucilage (1 part gum to 3 parts water) on the zinc plate (H).

2. Take a piece of the tissue to be cut of about ¼ of an inch thickness, and press it into the gum.

3. Fill the ether bottle (J) with anhydrous methylated ether, and push the spray points into their socket (E). All spirit must, of course, have previously been removed by soaking the tissue for a night in water. It should afterwards be soaked in gum for a like time before being cut.

4. Work the spray bellows briskly until the gum begins to freeze; after this, work more gently. Be always careful to brush off the frozen vapour which, in a moist atmosphere, may collect below the zinc plate. If the ether should tend to collect in drops below the plate, work the bellows slower.

5. Raise the tissue by turning the milled head (G), and cut by sliding the knife along the glass plates.

6. After use, be careful to wipe the whole instrument clean.

7. Should the ether point become choked, clear by means of a piece of fine wire.

8. The instrument is intended for use with methylated sulphuric ether.

9. In clamping the instrument to a table, or other support, care should be taken that the zinc plate is in a horizontal position. If the plate be not horizontal, the gum will tend to run to one side.

If, after the ether point has been cleared by the fine wire, it should still fail to act properly, it had better be returned to the maker for re-adjustment. For this purpose the spray points *only* need be sent.

At a temperature of 508 Fah., the instrument, if in

roper order, should freeze a quantity of gum, half an inch in iameter and about a quarter of an inch thick, in two minutes. he instrument will give the best results when worked in a *old* and *dry* atmosphere.

FIG. 227.

Mr. Swift is also the maker of several freezing micro- omes of good pattern, one form of which is shown for use ith ether in Fig. 227. The ice and salt section-cutter has one entirely out of use.

The student having become furnished with a microtome, his next selection must be a knife or razor, and in his choice he must remember that it is absolutely necessary for the *whole* of the back and edge to lie in the same plane—both must slide together over the top plate with perfect smoothness of motion. The knife or razor should be hollow-ground, flat-ground knives and razors being reserved for cutting wood or bone, where great strength is required. The cutting instruments must be kept to a very keen edge by use of a Washita oil-stone, combined with the application of a good razor-strop. It requires practice to put on the keenest edge. Perhaps a lesson from a barber would, on the whole, be a cheaper and speedier way of learning.

Mr. M. H. Stiles has described an arrangement by which the blade of the razor is kept from contact with the plate of the machine, by two small screw-clamps he terms "razor-guards" (Fig. 228). These slide on the blade, one being fixed at each end, and kept in position by a small screw in the upper side of each. They are $\frac{3}{8}$ths of an inch wide and are made from sheet brass $\frac{1}{11}$th of an inch thick. They ensure a smoother and more steady motion than by cutting in the old way, and the edge of the razor is preserved in much better condition, as it touches nothing but the substance to be cut.

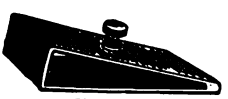


FIG. 228.

Let us now consider the cutting operation. If we wish to cut a section of the stem of the horse-chestnut (*Æsculus hippocastanum*), Fig. 131, say in a line with the axis of growth, we shall find it rather difficult, without first embedding in some agent to hold it firmly, and without undue pressure upon any one part. Formerly a mixture of equal parts of beeswax and olive oil was used for this purpose, and Professor Rutherford advises a mixture of five parts of paraffin with one of hog's lard. Both of these mixtures are

iable to become loose in the cylinder, often rotating during he cutting operation, and also "rising," and thereby spoiling he work by uneven thickness.

Mr. John Barrow has improved this bedding by making . mixture of naphthalin and stearine in certain proportions, rhich vary according to the temperature of the air and its onsequent behaviour under the knife. It can be mixed in uantity and kept in a large glue-pot, or even a stoneware ar or preserve-pot, and remelted by standing in hot water rhen required for use.

This mixture does not become loose in the well of the nicrotome like the paraffin mixture, and never "rises," so hat sections are easily cut of a uniform thickness.

Having soaked the stem in water, in order to soften it omewhat, it should be dried externally and dipped into the ollowing solution, which has been carefully filtered:—

Finest gum-arabic	60 grains.
Glycerine	5 drops.
Alcohol	10 drops.
Water	2 ounces.

The stem must then be withdrawn and allowed to drain upon lotting-paper until surface dry, when it is held in the fluid edding agent in the well of the microtome, until the aphthalin mixture has become hard, the plug having been reviously depressed, by means of the screw, to the depth equired.

When quite cold the cutting may be commenced, and the rst cut or two made with an ordinary razor and discarded, eing only intended to level down and to ascertain if all be ight. After levelling, the second cut with the section razor nould be examined under a low power, and if it is not thin nough more care must be taken with the subsequent uttings. After every cut, the necessary thickness must be ent up by means of the screw below, and if the cutting has

been done properly, *i.e.*, by a firm diagonal push from point to handle of the knife, keeping this also back and edge at the same time upon the cutting plate, the section will finally look like the illustration in Fig. 131.

During the cutting operation the knife or razor should be kept flooded with dilute glycerine or weak alcohol, and the section may be easily liberated from the knife by gently shaking in a basin of water. The glycerine and gum surrounding the sections dissolves after a few minutes, allowing them to fall to the bottom, while the naphthalin mixture floats on the surface of the water.

Most vegetable substances can be cut in this way, leaves and stems among the rest; but some will require preparation before inserting into the microtome. Those which are too hard must be soaked in water until soft enough to cut, or even immersed in boiling water if necessary. Excess of water may be abstracted by soaking in alcohol (methylated spirit) for a time, or by partial desiccation. Whenever it is required to eliminate resinous substances, methylated spirit will perform the operation, but it must not be forgotten that alcohol extracts the colouring matters from most vegetable substances.

Very good sections of certain woods can be made with an ordinary smoothing plane, such as is used by joiners. Deal may be cut in very thin ribands by this means, and when mounted dry, constitutes Dr. Carpenter's test for the colour correction of objectives.

A word of advice to the beginner may here be necessary: discard all sections not showing the leading features intended; they are worthless from a scientific point of view, yet it is strange how many there are in existence. Fig. 229 is an illustration of a section of a lichen thallus, and Fig. 230 a similar slice from the apothecium: mounted sections should show the details as well as these.

Animal tissues require different treatment. They are generally too soft and pulpy for immediate cutting, so require to be soaked for various periods in some hardening

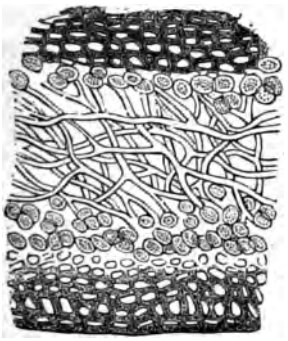


FIG. 229.

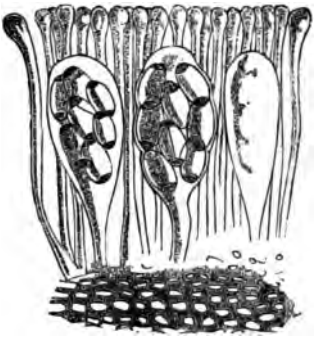


FIG. 230.

solution; while others, such as porcupine-quill, whalebone, and horn, require immersing in hot water to soften them.

For hardening animal tissues, alcohol, and chromic acid solution are most generally used, while some prefer bichromate of potash, or bichloride of mercury. Alcohol is perhaps the safest of hardening agents; it being a powerful abstractor of water, coagulating albumen, and acting also as a preservative agent.

Chromic acid is to be obtained in beautiful dry carmine crystals. It is more conveniently kept as a stock solution, 1 ounce being dissolved in 50 ounces of water, and diluted as occasion requires.

It must not be forgotten that tissues are easily overhardened and rendered brittle by prolonged immersion in chromic acid, and therefore the weaker a solution can be used the better. Some tissues require hardening by special

means. Rutherford advises brain to be soaked in the following solution:—

Chromic acid	15 grains.
Bichromate of potash	31 grains.
Water	43 grains.

Small pieces are first immersed for twenty-four hours in methylated spirit, drained on blotting-paper, and then soaked for five or six weeks in a large quantity of the above solution, changing it several times in that period. Adipose tissue may be hardened in methylated spirit; liver prepared for cutting by soaking in alcohol, commencing with weak methylated spirit and finishing in absolute alcohol; lung in chromic acid, muscle in chromic acid, as well as tongue, stomach, and spinal cord—the last in one part of the stock solution diluted with nine of water, as it is extremely liable to over-hardening. The actual operation of hardening may be performed in the following manner: Cut the substance—kidney, for instance—into pieces half an inch square, and about the length suitable for the well of the microtome. Place these in methylated spirit diluted with an equal bulk of water for three days, drain well upon bibulous paper, and then immerse in a solution of chromic acid prepared by diluting one part of the stock solution with seven parts of water. Allow the pieces to remain in this for three days, then pour it off and replace it by fresh. At the end of ten days a piece may be abstracted and a rough section cut, in order to see whether it has become sufficiently hardened; if not, the chromic acid must be again poured off and the pieces covered with fresh solution, making an examination of it with the razor every three days. Always harden insufficiently in the chromic acid, and when just under that degree required, take it out and put into methylated spirit to cleanse. Pour off the spirit every day and replace it with fresh until the excess of chromic acid has been

abstracted, known by the spirit remaining clear and colourless.

Injected kidney, and, indeed, most other *injected* tissues, must be hardened entirely in alcohol, as the chromic acid, being a powerful oxidiser, would in all probability cause a decomposition of the colouring matter. Kidney is often injected with Prussian blue. In this case it is as well to add a few drops of hydrochloric acid to the alcohol used in hardening.

The operation of cutting sections by means of Cathcart's freezing microtome has already been described on page 272. We may now mention how animal sections can be cut from a cereous bedding in the section-cutter, illustrated by Fig. 225.

The substance when taken out of the spirit must be placed on blotting-paper, and allowed to become surface dry. When this is effected it must be dipped into the gum solution before mentioned, and, after removal, is to be again laid upon the paper till dry.

The microtome having been screwed to the table, and the plug lowered by means of the screw, the melted mixture of naphthalin and stearine is to be poured into the well. When just about to set, the substance is inserted carefully, and held in position until the bedding has sufficiently solidified. After an hour or more, according to the temperature of the atmosphere, the substance will be in a fit state for cutting, and may be performed in exactly the same manner as described for vegetable stems.

The section knife is best kept in order on an ordinary razor-strop, with an occasional touch on a fine Washita oil-stone.

We now come to the preparation of sections of hard substances, such as bone, coal, rocks, and minerals generally. Some operators start with a fragment chipped from a

large specimen; but without doubt when several sections have to be cut, the best plan is to resort to what is termed slitting, being done by the microscopist himself or sent to the lapidary, who will cut slices for a very small charge.

If the operator desires a bench of his own, it would be advisable to have the slitter and laps interchangeable, so that they may run on the same centres. The woodcut, Fig. 231, will show how such a bench may be constructed.

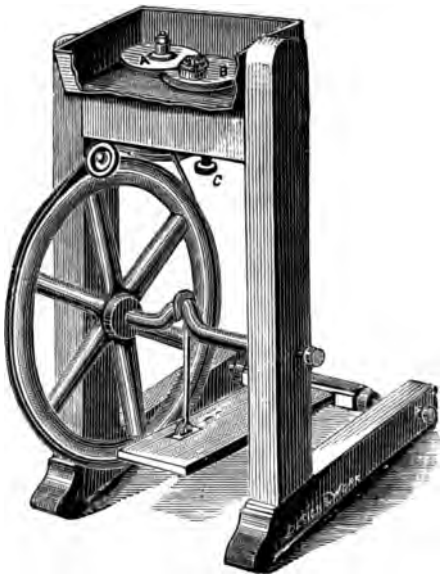


FIG. 231.

The slitter is a thin wrought-iron disc about 11 inches in diameter by about $\frac{1}{60}$ of an inch in thickness, and when used its edge is charged with diamond dust in the following manner:—Reduce a small splinter of diamond to a fine powder on a hard steel plate, then run a small quantity of tallow on to it, and mix thoroughly with the steel crusher. Press the tip of the longest finger into the mixture, bringing

away a thin coat. The slitter should then be moistened with petroline, and a bloodstone or agate pressed gently against it; at the same time the finger should be brought over the revolving disc, in such a position that the edge may just scrape the tallow off. This should be evenly distributed round the edge of the disc, which is readily done by rapidly touching it while revolving.

The rock, fossil wood, or other mineral, is now to be ground flat on one side, and firmly cemented by old balsam or marine glue (solid) to a glass slide, say 3 × 2 inches and ¼ inch thick. This can readily be slid along the guide-plate B, insuring perfect parallelism, the requisite thickness of each section being regulated by the screw C, which raises and depresses the guide-plate. The sections may be about $\frac{1}{16}$ of an inch in thickness, more or less, according to the subject, but the thinner they are cut, so much more labour is saved in the subsequent operations. The sound indicates when the slitter is cutting properly.

One side must be ground down on the leaden lap, which may be substituted for the slitting disc A in the bench shown in Fig. 231. This lap is about 10 inches in diameter and $\frac{3}{4}$ of an inch thick; it is used with fine emery and water for the first grinding, the final being performed with still finer emery upon a ground brass lap, preferably made to run in the same direction as the face-plate of a lathe—in fact, one of the cheap lathes shown in Fig. 232 will do exceedingly well for this work, and no doubt the ingenious student will be able to rig up a slitter and leaden lap to fit this form of lathe, which may be made a very useful tool by the practical microscopist.

In the grinding operation the student must remember that a *polish* is not necessary. The Canada balsam in which the section is mounted produces an apparent polish. All that is required is to produce a very smooth surface free from

scratches, and this is readily done with a brass lap and the finest emery procurable. The one side of the specimen having been brought into the above condition, is to be cleaned and firmly cemented with old balsam to the centre of a 3 × 1-inch glass slide. The balsam is dropped upon the centre and warmed over a lamp until, when cold, it is just possible to produce an impression with the nail. The balsam is then to be rendered fluid again, and the polished section

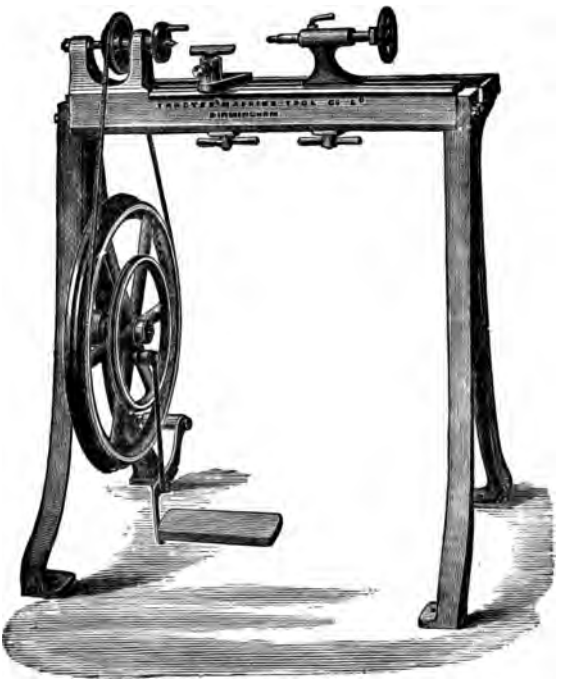


FIG. 232.

made gradually hot, when it is let down gently, taking care to avoid air bubbles, pressed down firmly, and then put aside to set thoroughly. After several days, the rough side may be ground down on the leaden lap with emery, and finished off

on the brass lap with the finest emery. The slide is now finished off by cleaning with spirits of wine and a camel's-hair brush, then dropping some fluid balsam upon the centre of the section, and covering in the usual way with thin glass. If the glass of the 3 × 1-inch slide be disfigured with scratches it may be covered with an ornamental cover-paper, sold by dealers in microscopic specialities. The foregoing method will produce good results for almost any kind of work; but, of course, there are simpler methods which may suit the student, requiring, however, much more labour. One of our friends grinds down roughly upon a grindstone or emery wheel, and finishes upon an Arkansas oil-stone. It is rather tedious work, but produces good results.

Mr. J. H. Jennings gives the following simple instructions:—1. Preparing sections of hard rocks: In the first place a thin chip must be procured by the use of a hammer. This chip should be about 1 inch square, and not more than $\frac{1}{8}$ inch thick; chips of sedimentary rocks may be thicker. Rub the chip down by hand with emery and water on an iron plate, until one side is perfectly flat. To remove scratches, next rub the chip on a glass plate with fine emery, and polish on a Water-of-Ayr stone; when quite smooth, wash it well and let it dry. Meantime place some old balsam on a glass slip, and warm it over a lamp until all the more volatile parts of the balsam evaporate, so that on cooling it becomes hard and tough. Do not let the balsam boil. When the balsam is properly hard, warm the chip gently over the lamp or on a hot metal plate, brush it over with a little turpentine, and re-melt the balsam; then lower the chip slowly into the balsam until it is cemented firmly and evenly by its flat surface to the glass slip. When the balsam is quite cold the chip is to be rubbed down on the iron plate with coarse emery until it is too thin to bear any further rough friction. With care, many rocks may be brought to

the requisite thinness on the iron plate alone, and will require little finishing. The necessary degree of thinness will vary according to the nature of the rock; but, as a general rule, most hard rocks must be cut thin enough to read through when placed on the page of a book. When the section will no longer bear the friction of a coarse emery, remove it to the glass plate, and grind it thinner with flour emery, and finally finish it off on the Water-of-Ayr stone. The slide, at the finish, will be disfigured by deep scratches from the emery, and the section must be transferred to a clean slip. Warm the section enough to melt the balsam, and push the section off with a needle into a cup of turpentine, and wash carefully with a small brush. Now pour a little balsam and benzol solution on the clean slip, place the section upon it, add a little more balsam, and cover.

2. Preparation of soft rocks and sedimentary rocks generally: These are prepared and mounted in the same way as hard rocks, *but no emery is to be used;* they must be ground down and finished on the three stones mentioned above. Some very friable rocks will require a preliminary hardening by immersion for some days in a solution of (1) balsam in benzol—the balsam must be first baked in a cool oven, until, on cooling, it becomes hard and brittle; then dissolve it in benzol; or (2) in a solution of shellac in alcohol. This is, perhaps, the better of the two. When the chip has remained long enough in the solution it must be dried in a warm place. Sedimentary rocks, as a rule, do not require to be cut as thin as igneous rocks, so that they may be left on the grinding slide, as it will not be scratched.

One thing is absolutely necessary in either method, that is, to get rid of *all* air bubbles in the balsam attachment, as if any are left, the section is sure to wear into holes and break.

Sections of Echinus spine may be cut in the same way as

bove, or with a fine saw improvised from a thin clock pring, and the slice ground down by rubbing on a fine level urkey hone, and when thin enough should be cleansed ith water and a soft camel-hair brush, dried by immersion n alcohol, passed through benzol, and finally mounted in alsam and benzol, or dry, if required, when it is ready for bservation.

Hard rocks, as a rule, are easier to prepare than soft nes. The latter should be soaked in turpentine, and hen in balsam and benzol, afterwards being heated till quite ard.

Coal may be cut and ground into sections in the following anner:—After careful selection of a piece free from cracks, prism is to be cut $\frac{1}{2}$ an inch or $\frac{3}{4}$ of an inch square. Slices of his, as thin as possible, may now be cut with a fine saw, and ne face rubbed flat upon a slab of pumice-stone kept well etted with water, and finally rubbed smooth upon a Water-f-Ayr stone, water being continually applied to it. The olished surface is now cemented to a 3 × 1 glass slide with arine glue, taking special care not to include air bubbles, nd the slide put by to set for some days. When this has aken place, the slice may be rubbed down on a flat piece of oarse gritstone with plenty of water until almost thin nough to show the structure, and when arrived at this stage, inished off first on the pumice-slab with water, and finally on he Water-of-Ayr stone.

The study of coal sections gives us much insight into the ormation of that peculiar substance. Fossil fungi have been ound in coal, and described by Mr. Worthington Smith as elonging to the genus Peronospora, and the plants have heretore been called Peronosporites. They are shown in ig. 233.

Bone and teeth are generally cut with a saw, and afterwards rubbed down with a hone and water. Dr. Beale

objects to this method, as filling up the lacunæ and canaliculi with debris, and advises cutting a thin section with a sharp knife, afterwards staining with carmine.

A very good, and, at the same time cheap, section-cutter for rocks and minerals was described by J. Tertius Wood in the "Northern Microscopist." It consists of a strong wooden box (A) (Figs. 234, 235) without bottom, with the front side taken off to demonstrate the details inside, consisting of a solid wooden fly-wheel (B) of sufficient thickness to take a

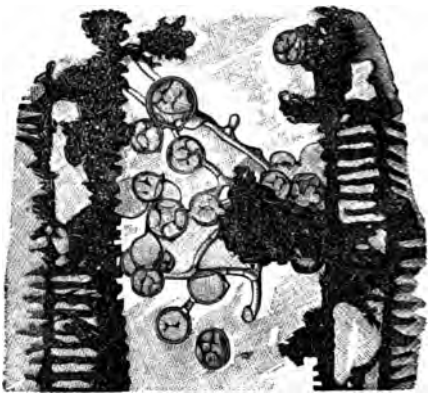


FIG. 233.

tolerably broad strap, and its shaft (C), which at one end is fixed into a piece of wood stretched across the bottom to receive it, and at the other end is the handle. This wheel turns a small drum, also of wood, and its shaft (D), on which is attached horizontally an ordinary tinned iron disc, about nine inches in diameter, fixed on like a circular saw by large washer and nut. It is safer to have the upper part of the shaft (D) made of brass, as the perfect adjustment of the disc constitutes the main thing.

In one corner is fixed an iron standard, running the entire depth of the box to ensure its perfect rigidity, turned to receive one end of the clenching rod, which should rotate

freely and evenly. To the clenching iron (K) is attached an iron plate (L) by two thumb-screws (HH). Between these two screws and towards the bottom is another thumb-screw, which does not penetrate the plate (L), but acts as a check to prevent that plate from dipping downwards and imper-

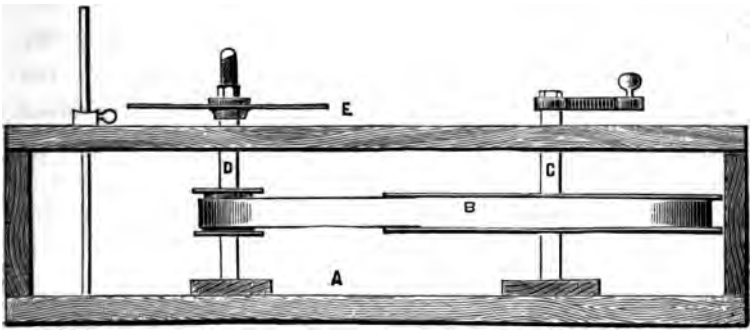


FIG. 234.

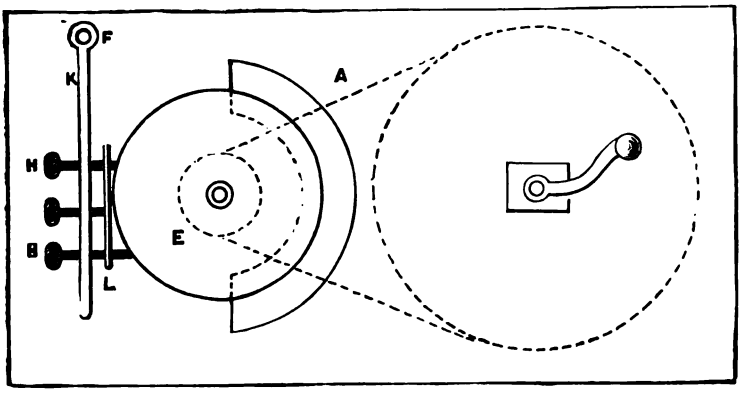


FIG. 235.

fectly holding the stone, which is placed between these two plates in front of the disc (E). By means of this a glass slip can be securely fixed without slipping, and if the stone to be cut is delicate, it can be cemented to the slip and a slice taken off *in situ* ready for grinding.

Round the disc is placed a movable guard-box of tin, to prevent the water and dirt from flying on to the operator, and preserving the utilized emery, which comes in use when grinding.

For working the machine—get the specimen as square as convenient, and clench it at the required angle between the plates in front of the disc. With the right hand turn the handle, and hooking the little finger of the left round the clenching rod, thus exerting gentle, even, and continuous pressure of the stone against the disc, at the same time dropping at intervals some *red* emery powder stored between the finger and thumb. The emery is thus taken round with

FIG. 236.

FIG. 237.

the disc, and is pressed into the soft metal, thus making a sort of file. Water must be allowed to drop on the disc, but not too copiously. About a square inch of limestone may thus be cut in about twenty minutes, harder stone taking a proportionately longer time, but by exerting a greater speed nearly twice as much may be done in the same time.

For grinding, remove the cutting and substitute the grinding disc, as shown in Figs. 236, 237, where a circular ring of lead is fastened to a plate of iron. Make the cut piece of stone into a perfect plane on one side by rubbing it on a perfectly even whetstone, and cement that side on to a glass slip in the usual manner. When set, seize the end of the slip between the finger and thumb of the left hand, and having brushed some of the wet emery mud out of the tin

guard round the lead rim, set the machine in motion, and gently press the section lengthways across it. If the stone be soft, it is unadvisable to grind with emery to transparency, as the powder becomes incorporated with the stone, but finish by giving a circular motion on the whetstone. After cleansing with turpentine and warm water, mount in the usual way. Any number of parallel sections may be cut by simply raising the clenching arm, and tightening the ring underneath by means of a thumb-screw.

Dissections and section-cutting are generally looked upon by the student with awe, as being exceptionally difficult. This is not the case: with proper care and perseverance almost anyone may become an expert dissector or section-cutter, and it is to be hoped that whoever purchases a microscope through reading these pages will endeavour to do some useful work, and not be content with mounting a few crystals for the polariscope, or with soaking an insect in potash, and mounting in balsam, believing that is all that need be done with it.

CHAPTER XIII.

THE DELINEATION OF OBJECTS.

WE have now to dilate upon the importance of sketching everything of interest the observer may see under the microscope.

Dr. Beale says: "The student cannot too soon try to delineate what he demonstrates. He will teach himself to observe the more accurately and the more quickly if he record the results of his work in pencil sketches. A truthful drawing of what a man has recently seen may be compared with drawings made 100 years hence; and although the means of observation will be more perfect than they are at present, such comparisons may be useful in many ways, and especially in preventing erroneous conclusions from becoming popular."

It cannot be too strongly impressed upon the reader, of the advisability of frequent practice with the various means devised for this purpose. Microscopical contributions to our scientific papers, communications to societies, and even the results of our everyday observations, all become more interesting if illustrated in some way or other.

There are many methods by which microscopical objects may be delineated. They may be roughly drawn by means of pencil and paper, guided by the eye alone, or assisted with

the use of a circle of glass ruled into squares, made to rest upon the diaphragm of the eye-piece; the paper, in this case, may also be faintly ruled into squares, and by this means, especially after a little practice, very accurate drawings can be made. Enlargements and reductions can also be just as easily drawn. A glance at Figs. 238, 239 will illustrate this. The former represents what the observer is supposed to see upon looking down the eye-piece of the microscope; while the

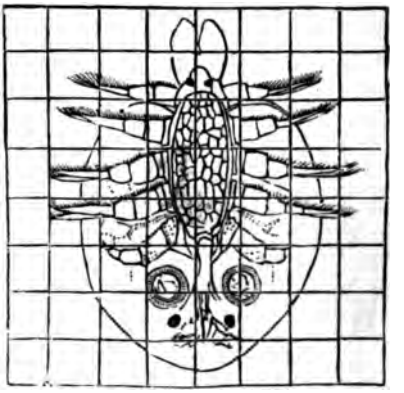


FIG. 238.

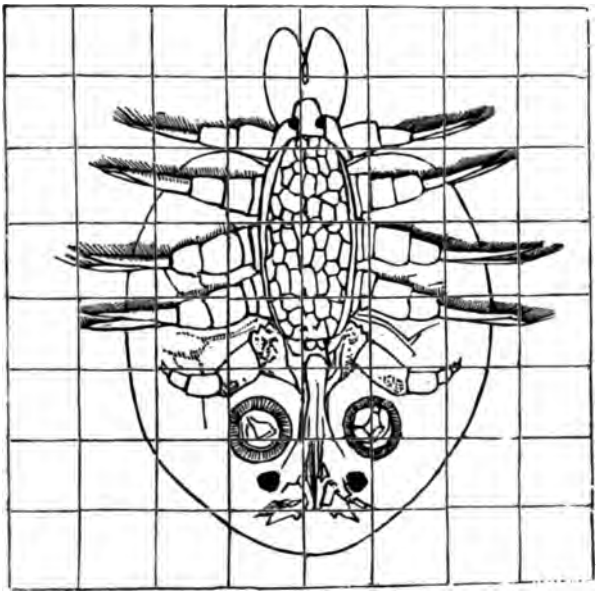


FIG. 239.

latter delineates the figure produced by the pencil upon the ruled paper.

Another method for the delineation of objects is by the use of the camera-lucida or neutral tint reflector, which by a little practice becomes a very accurate process. The ordinary camera-lucida consists of a prism, and apparently throws down an image of the object upon the paper below it.

Fig. 240.

This is the same with the neutral tint reflector; it is made of a small piece of plate glass, slightly coloured and arranged at an angle of 45° with the eye-piece; it is shown in Fig. 240.

There is a fault with nearly all reflectors hitherto made; the glass is too thick, and consequently two or more images are formed of the object, which come into view by a slight movement of the head. To remedy this, a writer in "Science Gossip" proposed a head-rest, but even this may be dispensed with if the glass used be of the same thickness as an ordinary cover, such as is used in the mounting of objects. In the construction of a reflector for drawing purposes, it is only necessary to hold a thin glass cover at an angle of 45° with the eye-glass, in such a position that the centre of the cover coincides with the optical axis of the microscope. Surely students interested may manage to do this for themselves.

When using either the camera-lucida or the neutral tint reflector, the cap is removed from the eye-piece and the accessory placed in its stead, the microscope arranged horizontally, and the paper placed under it upon the table, as shown in Fig. 241.

The instrument shown is one of Messrs. Ross's make, and is nicely balanced in all positions. Messrs. Swift and

Son place a stop on most of their stands, so that it is easily known when the body is either horizontal or vertical.

The most important point to be remembered in the use of these reflectors is the proper management of the light. Perhaps the student will fail at first in seeing *together* the pencil and the object, but this difficulty will vanish upon securing the proper illumination. The light must not be concentrated too strongly upon the object, neither should the paper be placed entirely in the shade, the student soon arriving at the happy medium after a few trials.

FIG. 241.

In order to obtain the same degree of amplification upon the paper as appears when looking down the tube of the instrument, the paper must be placed at the same distance from the camera-lucida as that accessory is situated from the objective front; but the magnification is generally expressed at a distance of 10 inches.

Another form of this instrument is Nachet's, for use with

vertical microscopes, with the ordinary pattern when used vertically with immersion lenses, or in any other position of the instrument.

A diagram of this form is shown in Fig. 242, from which the reader will see that it consists of a prism of nearly rhomboidal form placed with one of its inclined sides over the eye-piece of the microscope. To this is cemented a segment of a small glass cylinder, constituting the complete apparatus. This enables sketches to be made with the microscope body disposed vertically with the greatest ease, as, although the ordinary camera-lucida or neutral tint reflector can be used with the microscope in this position, still, drawing from it in this way is not a comfortable operation.

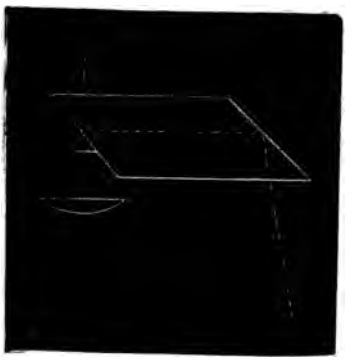


FIG. 242.

FIG. 243.

Abbe's camera-lucida is shown full size by Fig. 243. The drawing surface is made visible by a double reflection from a large plane mirror and from the silvered surface of a small

prism in the visual point of the eye-piece. The microscopic image is seen through an aperture in the silvering of the prism. The brightness of the paper is regulated by smoke-tinted glasses, made to fit into the prism mounting. This

FIG. 244.

camera-lucida is specially adjusted for the No. 2 Huyghenian eye-piece; mounted on this and fixed by the clamping screw, the mirror only requires to be turned to the proper position and it is then ready for use.

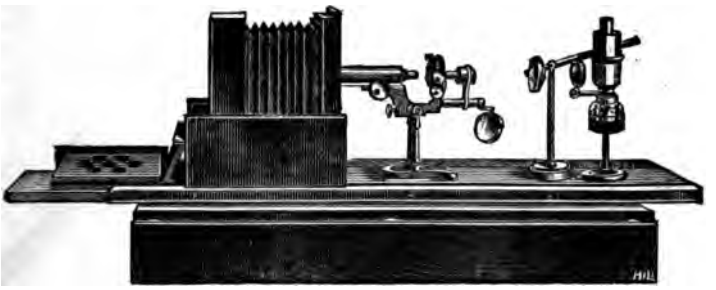


FIG. 245.

There is another method by means of which microscopic objects may be faithfully delineated, and that is by photo-micrography. This art is usually considered a difficult one, and when we are confronted with the fact that nearly all who

have written anything upon the subject have advised the use of a host of complicated paraphernalia, it is scarcely surprising that such an opinion should have gained ground. The vast array of apparatus, condensers of peculiar construction, blue cells, heliostats, and even to a special room set apart for the camera alone, must have been quite sufficient to frighten anyone with only their evenings at leisure.

An ordinary microscope and photographic camera, as shown in Fig. 245, is all that is absolutely required for this work, though with some it may be more convenient to use the apparatus illustrated by Figs. 244 and 251.

The camera proper is made to remove from the base-board, but this is for portability only. The base-board should project for some distance in front of the camera to carry the microscope and illuminating apparatus, as in Fig. 245, so that the whole may form a solid continuous base. This is necessary, as well as a good firm table upon which to place the apparatus, and this must be quite free from any vibration to insure perfect sharpness of the image.

Of course, the length of the base-board and of the camera body, when extended, must vary with the amplification required. The diameters to which an object is enlarged in the camera (Fig. 244) when the sensitive plate is at a distance of 36 inches from the object, may serve as a guide in this direction.

Designation.	Diameters.	
	Without Eye-piece.	With the A Eye-piece.
4-inch	12	36
2 „	21	63
1 „	37	110
½ „	80	240
¼ „	173	520
⅛ „	360	1000
1/15 „	530	1600

If the operator prefers to work with the eye-piece in addition to the objective, the camera need only be a short one, say extending from four inches to twelve, the apparatus being arranged as shown in Fig. 245. At this latter distance he will get the same degree of amplification as when looking down the tubes of his microscope, and the author has but few doubts whether, after all, despite adverse criticisms, this is not the simplest and cheapest plan. He has both taken, and seen taken, photo-micrographs of the same subjects, with and without the use of eye-pieces, and could not discover any difference between them.

There is more light lost when working with the eye-pieces attached, but in these days of extra-sensitive plates this is certainly no disadvantage with the lower and medium powers.

The microscope employed may be of any ordinary kind, monocular preferably, and if provided with a means of shortening the tube or body so much the better. The tube should be lined with velvet, to prevent central flare, and the instrument provided with a coarse and fine adjustment, the latter answering the helm when turned either backwards or forwards; but more particularly must it be sensitive to very slight movements of the milled head when *withdrawing* the objective from the stage.

Dr. Woodward states "the objective selected should always be specially corrected for photography." The author is not able to agree with Dr. Woodward on this point; no doubt it is very convenient to have one so corrected, but it is by no means imperative. To those as yet uninitiated in the mysteries of the photographic art, it may be necessary to say that the *visual* and *actinic* foci often (though not necessarily) lie in different planes, and therefore an objective which gives perfect definition *to the eye* will often produce a blurred and indistinct image upon the sensitive plate. This

may be remedied in withdrawing the objective, by turning the milled head of the fine adjustment; it cannot be told beforehand what allowance is necessary, the only method is by trial and error; some objectives require no correction whatever, while others which necessitate considerable movement from the object, can be made to yield just as perfect results.

The best way to arrive at the necessary correction is to take a picture at the best *visual* focus, develop, fix, and dry in the usual manner, and then withdraw the objective from the stage by means of the fine adjustment until the image appears on the ground glass about as indistinct as it does in the negative. This will probably be the amount of correction required, and one or two trial plates will decide the question. For powers up to the half-inch the proboscis of the fly is a good object to experiment with; a photograph of this preparation, taken with a Ross's 1¼-inch, showed that its actinic focus was identical with its best visual, while a 2-inch, by Dancer, required one complete revolution of the milled head of the fine adjustment.

Mr. G. J. Johnson, in a paper read before the Manchester Photographic Society in 1883, described a very simple focussing arrangement for the microscope then used for photomicrography.

On the shaft of the fine adjustment screw a short split brass tube D, Fig. 246, half-an-inch long, is made to slide stiffly, to which a stout wire pointer, three or four inches long, is soldered. A semi-circle of cardboard, E, having its centre coincident with the axis of the fine adjustment screw, is placed behind the pointer, and marked in its circumference with degrees. The split tube allows of the entire revolution of the adjustment screw, whilst the pointer acts as an index through 90° of arc, and records with exactness any slight alteration in focus.

This is shown by Fig. 246 and Fig. 247, the portions B F and G being attached to the base-board of the camera, and used for focussing by means of the milled head, G, the

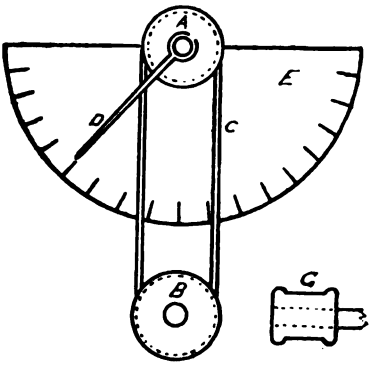


FIG. 246.

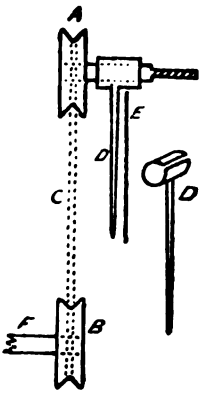


FIG. 247.

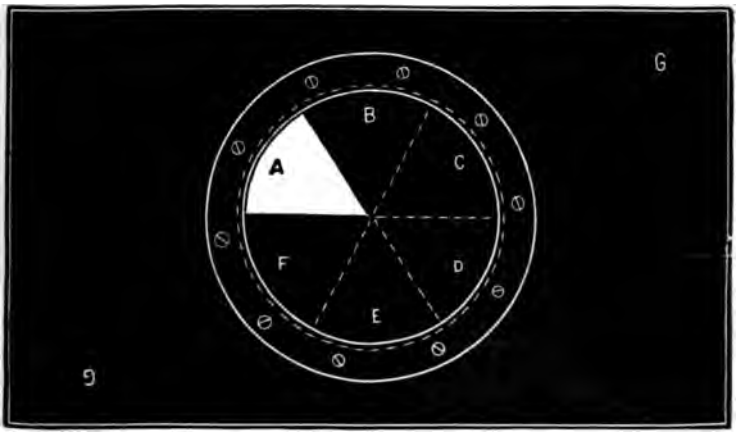


FIG. 248.

fine adjustment screw, A, and the small pulley, B, being connected by an india-rubber band, C.

The various trials for actinic correction, as well as for the exact exposures required, may be most readily made by means

of an apparatus devised by Mr. Shipperbottom, and shown in Figs. 248 and 249.

FIG. 249.

It is a revolving shutter (Fig. 249) with a diagonal opening, H, to be fixed just in front of the dark slide. Six different exposures may be made on one plate, each with a slightly different amount of adjustment of fine screw, thus on one plate the different amount found necessary to give the sharpest possible focus may be found. It will be quite clear also that you secure a record of the depth of focus and flatness of field of objective under examination—a transverse section of the stem of the lime tree will be as good as any. First focus the object as usual on the focussing screen, which should be rubbed over with a little oil. Now put the revolving shutter in position with the diagonal opening at A, and give the first exposure. Shut off the light, close the dark slide, then turn the revolving shutter to F; make slight alteration with fine adjustment screw, give second exposure, and so on through six different exposures.

The microscope stand employed by the author possesses a fine adjustment, moving the objective $\frac{1}{100}$ of an inch for each revolution of the milled head, and is divided into twenty parts. His objectives require the following corrections:—

Designation.	Maker.	Air Angle.	Correction.
4-inch...	Browning	9°	0·0100 inch
2 ,, ...	Dancer	14°	0·0050 ,,
1 ,, ...	Tolles	32°	0
½ ,, ...	Browning	60°	0·0005 ,,
¼ ,, ...	Wray	118°	0
⅛ ,, ...	Browning	140°	0
1/15 ,, ...	Dancer	170°	0

Ordinary ground glass is too coarse to focus upon; two surfaces should be rubbed together with a little of the finest emery and water, or a focussing glass may be prepared by coating an ordinary glass plate with the following varnish:—

Gum mastic	40 grains
Gum sandarac	160 grains
Ether	4 oz.
Benzol	1½ oz.

The correct focus may be obtained by the eye alone, though this is difficult, especially with high powers; and, therefore, it is advisable to employ a magnifier of some description —an engraver's lens or an ordinary eye-piece with the eye-glass removed, as shown in Fig. 250. The field lens is placed next the eye and the open brass tube slid in a parallel direction over the glass focussing screen.

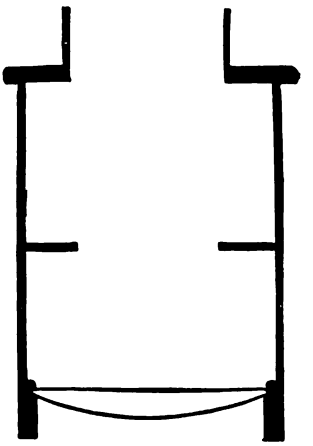


Fig. 250.

The author now uses a much improved method for focussing:— Removing the ground glass slide, another is substituted of mahogany, but pierced with a series of seven holes, into each of which the ordinary A eye-piece may be fixed. The thickness of the slide is such that when the eye-piece is pushed in as far as it will go the diaphragm lies in the same plane as the ground surface of the glass slide. To anyone accustomed to focus by the old method, the present system will be found a considerable improvement, it being *easy* under these conditions to obtain a sharp focus with an ordinary paraffin lamp when using the $\frac{1}{16}$ objective. (See Fig. 245.)

The source of illumination may be the ordinary microscopical paraffin lamp, as the light from it is much more

actinic than that of gas, and for all powers below the ½-inch it is only necessary to use the bull's-eye condensers as shown in the figures.

A very intense illumination for photo-micrography may be obtained from paraffin oil, by using it in a triple-wick lamp, such as the Triplexicon or Sciopticon, with a condenser parallelising the rays proceeding from it, as shown in Fig. 251. These parallel rays are again to be converged by a convex lens of 3 inches diameter and 10 inches focus. Such an arrangement, as used by the author, is shown by Fig. 251, and

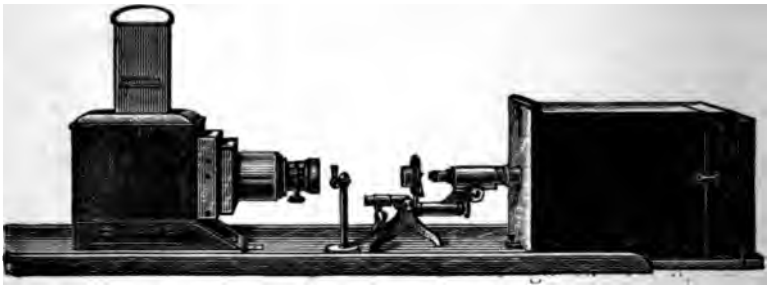


FIG. 251.

by means of it the relative lengths of exposure have been very much shortened.

With the objectives already alluded to, the sensitive plate being at a distance of 24 inches from the object, with an ordinary paraffin lamp and bull's-eye condenser of 3 inches focus, the shortest exposures for good pictures, "Instantaneous" dry plates, have been found as follows:—

Designation of Objective.	Subject.	Exposures.	
		By Method Fig. 192.	By Method Fig. 193.
4-inch ...	Wing of blow-fly	6 seconds	3 seconds
2 ,, ...	Proboscis of do.	60 ,,	10 ,,
1 ,, ...	Do. do.	70 ,,	20 ,,
½ ,, ...	Glass crystal	60 ,,	30 ,,
¼ ,, ...	Section of deal	7 minutes	2 minutes
⅛ ,, ...	Podura scale	10 ,,	2½ ,,
1/16 ,, ...	*Pleurosigma attenuatum*	15 ,,	3 ,,

Dr. Carl Seiler, in a communication to the "American Journal of Microscopy" upon this subject, seems to prefer wet plates to dry ones, and adduces arguments in support of his views, one of which is cheapness. The cost of plates by the wet or dry process is so small that this item need not enter into the calculation; moreover, dry plates are rapid, so convenient, and with good manipulation give such excellent results, that the author pleads for the use of them exclusively in producing negatives.

There are many kinds of dry plates to be met with in the market, nearly all of which are now prepared from gelatine emulsion. Mawson and Swan's, having been extensively used by the author for this kind of work, deserve commendation as being of good and uniform quality.

Do not over-expose these gelatine plates, or you will get nothing but thin and misty images. From some cause, those exposed to gas or lamp-light do not *commence* to develop so quickly as those exposed to sunlight; but if time is given, and they are not over-exposed, perfect pictures may easily be obtained. The time of exposure in the camera depends upon the quality of the light, *but more perhaps upon the nature of the object than anything else;* for instance, a section of the kidney of a horse required twenty minutes' exposure with the argand gas and bull's-eye condenser, while a crystal of glass, under the same conditions of light and amplification, required but one minute.

For ascertaining the exact exposures and the corrections required for the difference in actinic and visual foci, the beginner is strongly advised to keep a bound record of his failures as well as prints from *all* his negatives, with full particulars respecting them; they are great helps in photographing new subjects.

Mr. Dancer, of Manchester, Dr. Maddox, Mr. Shadbolt, the late Dr. Redmayne, of Bolton, Mr. Wenham, and

several others have been the most successful photographers of microscopic objects; not forgetting, however, Colonel Dr. Woodward in America, as most of the photographs and nearly the whole of the literature of this country has emanated from him.

Dr. Woodward has described his method with sunlight and a heliostat, from which we gather the following :—

The microscope being placed on a shelf at the window of the dark room, and its body made horizontal, the achromatic condenser is illuminated by a solar pencil reflected from a heliostat (Fig. 252) upon a movable mirror outside the shutter, and thence into the dark room. No ground glass is used,

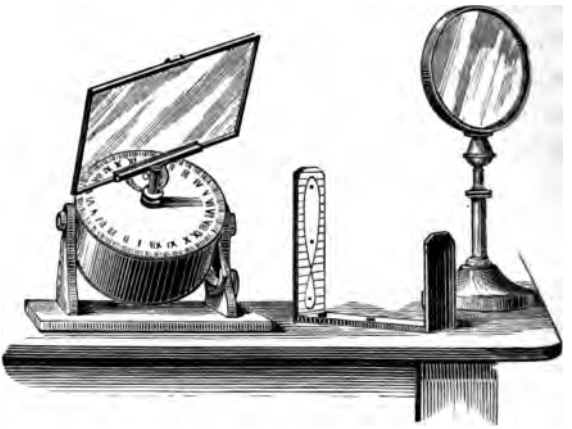


Fig. 252.

but a lens, mounted in a suitable tube, is fixed in the opening of the shutter through which the solar pencil enters. This lens is an achromatic combination about 2 inches in transverse diameter, and of about 10 inches focal length. It is placed at such a distance from the achromatic condenser that the solar rays are brought to a focus, *and begin again to diverge*, before they reach the lowest glass of the achromatic condenser.

All trouble from the solar heat is thus completely avoided. So successfully may this be done, that it is possible to obtain light enough to give distinct vision and admirable definition on the cardboard screen with 5,000 diameters, while the heat is so slight that the drop of water used with the immersion lens does not require renewal oftener than about once in two hours.

For anatomical preparations requiring for their display from 200 to 500 diameters, the $\frac{1}{8}$-inch objective may be used without an eye-piece, obtaining the precise power desired by variations in the distance of the sensitive plate from the stage of the instrument. Dr. Woodward gives the preference to immersion objectives, the corrections of which are generally well suited to photographic requirements.

With a one-eighth objective and the arrangement above described, the field is so brilliantly illuminated that the eye cannot safely be permitted to look down the tube. The image is therefore received on a piece of white cardboard, and, sitting by the microscope to make the adjustment, the card is viewed with both eyes, precisely as in the case of the ordinary solar microscope.

When all is satisfactory, an ammonio-sulphate cell is inserted between the large lens and the achromatic condenser, and the velvet hood, which prevents leakage of light from about the microscope into the dark room, is drawn down.

Dr. Woodward finds it best to use the naked objective without eye-piece or amplifier, and not as a rule to fix the sensitive plate more than 3 or 4 feet from the stage of the microscope; but this, of course, will depend on the amount of penetration required, for which refer to p. 178. A one-eighth objective may be conveniently employed to obtain powers of from 200 to 500 or 600 diameters; a one-sixteenth for higher powers, up to 1,200 or 1,500 diameters.

It occurred to him that for such short exposure the heliostat might be dispensed with, and on trial without it, found that a large right-angled prism, used in the position of total reflection, or even an ordinary mirror, gave excellent results; the exposures being even shorter than when the heliostat was used, since there was but a single reflection. Under ordinary circumstances the heliostat appears desirable for general use since, the solar pencil being thrown in a constant direction, the trouble of adjusting the illumination of a series of objects is considerably diminished; but equally good pictures can be produced without it, even with very high powers, a circumstance of considerable interest where motives of economy preclude the microscopist from procuring this convenient instrument.

The time of exposure required for the production of pictures magnified 500 diameters or less was about a second. With higher powers it increases, varying with the management of the achromatic condenser. For 4,000 diameters as much as 25 seconds is sometimes required.

In using the electric light it is necessary to render the divergent pencil proceeding from the carbon points as nearly parallel as possible, by means of the condenser usually supplied with electric lamps for this purpose, and then introduce into the parallel pencil, instead of a ground glass, the very same condensing lens described above for the process with solar light. The image is received primarily for focussing on a cardboard screen, and the remaining details do not differ from what has been related above. The time of exposure does not exceed a single second for 400 diameters, and the sharpness of the pictures is excellent. For well-made tissue preparations, however, the best work obtainable with the electric light is so similar to the best attainable by sunlight, used as above described, that it is scarcely necessary to take the trouble to set up the battery and work the electric lamp,

unless it was desirable to work at night or in unfavourable weather.

For the production of the electric light, Duboscq's lamp was used, set in motion by a battery of fifty small Grove's elements, and with this source of light photographs can be taken with any power with which pictures are possible in sunlight. The rubber cups of the battery were $4\frac{3}{4}$ inches high, $3\frac{1}{4}$ inches wide, and 2 inches thick; the platinums $5\frac{1}{2}$ by $2\frac{1}{8}$ inches, and weighed about 60 grains each. The zincs were bent on themselves so as to present a part of their

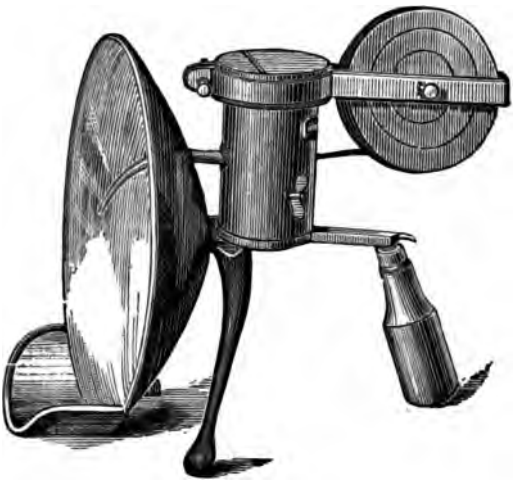


FIG. 253.

surface on each side of the platinums, and weighed when new about a pound each.

The electric light, as hitherto employed, has been a cumbrous, expensive, and troublesome process, which certainly could not be employed by the majority of persons wishful to practise photo-micrography. For those who may have to use high powers, and to whom daylight is not accessible, the magnesium light seems to possess advantages.

For this process a good lamp is necessary, and the one shown in Fig. 253 is the best of its kind.

In describing the magnesium modification of photography, as applied to the amplification of microscopic objects, Colonel Woodward compares the light given off by a good magnesium lamp to white-cloud illumination of the best character.

Without the use of ground glass, this light serves admirably for the production of photographs of soft tissue with any power under a 1,000 diameters. The light being composed of a mixed pencil, with rays passing in all directions, there are no interference phenomena; but for the same reason, on the Nobert's plate and many test-objects, the results are inferior to those produced by the sun or by the electric light; with powers much higher than 1,000 diameters, however, the time of exposure becomes inconveniently long.

To produce negatives of tissue-preparations with 500 diameters it required exposures of about three minutes. Other powers require proportionate exposures. Magnesium wire now costs only 2s. 6d. per ounce; two ounces will, with care, answer for three or four hours' constant work, and ought to produce from twelve to thirty negatives, in accordance with the difficulties of the subjects to be represented.

The method of photographing such objects as *Amphipleura pellucida* and Nobert's test-lines has also been described by Dr. Woodward in a letter to M. Deby, a vice-president of the Belgian Microscopical Society. Such objects as the *A. pellucida* require the use of very oblique light, which is obtained in the following manner:—A parallel pencil of solar rays, from the heliostat and plane mirror, is intercepted by a blue cell and diaphragm, which only allows a circular pencil of half an inch diameter to pass. The light enters parallel to the optic axis of the microscope,

placed in the usual position for photography, but at the lateral distance to the right or left of 3 inches. If the light is intercepted by a large achromatic prism, of a focal length of about 3 inches, the desired obliquity can be obtained without difficulty. The best result is obtained when the rays are concentrated to a focus upon the object, and it is indispensable that the stage of the microscope be as thin as possible. The illumination thus produced is in general sufficient to produce negatives by the wet process up to 2,500 diameters with three minutes exposure. Fig. 145 shows that difficultly resolvable diatom, the *A. pellucida*, under an amplification of 960 diameters.

After the sensitive plate has been exposed the necessary length of time by either of the foregoing methods of illumination, it is ready for development. It is, therefore, taken into a room from which every trace of actinic light has been excluded, and there the picture is brought to view by the use of the developing solution. Nearly every photographic manipulator has his own way of developing, though the general principles are the same, but that given by Messrs. Wratten and Wainwright for their instantaneous dry plates will perhaps be found the most suitable for photo-micrographical purposes.

STOCK SOLUTION A*.

Ammonia liquor, *fort.*	1 oz.
Potass bromide	60 grains
Water	2 oz.

DEVELOPER.

Pyrogallic acid	6 grains
Stock Solution A*	20 drops
Water	2 oz.

Lay the exposed plate in a dish of cold water to soak while the pyrogallic acid is mixed. For each ¼ or 5 × 4 plate use 6 grains of pyro., diluted with 2 oz. of water.

First pour off the water from the plate and apply the pyro solution, then add five drops of stock solution A*, and keep this weak developer on the plate until the highest lights are pretty well visible; then add from 15 to 20 drops more of A* to finish development. By this method more of the film is employed and greater density is obtained. Whenever any of solution A* is to be added to the pyro solution it should be first dropped into the developing cup, and then, if the solution which is in the dish is poured back to the cup, a perfect admixture will be the result, without the necessity of stirring. With correct exposure full printing density may be easily attained with the alkaline pyro alone. No plate is supplied that will not bear 100 drops of stock solution A* without fog, if the correct exposure has been given. One thickness of deep ruby glass is unsafe for the dark room; two, at least, are necessary. Should any discoloration of the film appear after the negative has been fixed and washed, it may be cleared away with a weak solution of perchloride of iron and water (about the colour of pale sherry), the plate afterwards to be washed thoroughly.

Ferrous Oxalate Development.—Where it is not expressly stated to the contrary, Swan's plates may be developed either with ammoniacal pyrogallic acid or with ferrous oxalate; but it occasionally happens that one and not the other of these developers must be used. When that is the case, the fact is stated on the label of the packet.

Ferrous oxalate developing solution is best made as follows:—

SOLUTION A.

Neutral oxalate of potash	6½ oz.
Water	25 oz.

SOLUTION B.

Sulphate of iron	2½ oz.
Water	7½ oz.
Sulphuric acid	1 to 2 drops.

The two solutions A and B are kept in stock, and when required for development three volumes of A are mixed with one volume of B.

It is better to mix and use what is immediately required for the development of a single plate, and not to use the same solution for developing several plates. The two stock solutions keep well, therefore any quantity of these can be kept ready for mixing. The sulphuric acid is not essential in the formula referred to; it can be omitted.

The bromide of ammonium solution may be added to the developer as a restrainer, to counteract over-exposure, in the same manner and under the same conditions as in development with pyrogallic acid and ammonia.

This ferrous oxalate developer should be used in a flat bath, precisely in the manner described for alkaline pyrogallic acid development. During the operation the solution must be made to wave to and fro over the plate, and the development continued until the required density is obtained. This will occupy about three minutes, as a rule. The addition of bromide affects most powerfully the rate of development and its character, making it slower and increasing the contrast of light and shade. For copying engravings, &c., where only black and white is to be printed, the proportion of bromide of ammonium may be advantageously increased.

If, during development or fixing, any loosening or blistering of the film should be observed, the plate must be immediately rinsed in water and put into a solution of alum (1 oz. to 1 pint of water). After allowing to remain five minutes in the alum solution, it should then be finished as usual after this treatment; and as all plates of the same packet will probably be alike, it will be advisable with each of these to use the alum bath after development, without waiting for the appearance of blisters or loosening of the film.

The image generally makes its appearance in about a minute, the deposit gradually increasing in intensity until the development is finished. It is then to be well washed and fixed in the following solution:—

Hyposulphate of soda	4 oz.
Water	20 oz.

In cases where, from over-exposure, it has been impossible to obtain density with the alkaline developer, a most careful washing should be given after fixing, with a view to remove the last trace of ammonia.

Intensification can then be effected with acid pyro. and nitrate of silver, or with protosulphate of iron and nitrate of silver. The author has a decided preference for the latter, and uses the following formula, viz.:—

A.

Protosulphate of iron	15 grains
Gelatino-acetic acid solution (as described below)	40 drops
Water	1 oz.

B.

Nitrate of silver	10 grains
Acetic acid, glacial, 50°	10 drops
Water	1 oz.

The gelatino-acetic acid solution is compounded as under:—

Gelatine	15 grains
Acetic acid, glacial, 50°	3 drachms
Water	5 drachms

and it is well to prepare a stock of this, and also of A, as they are both better for keeping.

To proceed. First flood the plate with water and then with a solution of iodine and iodide of potassium, of the colour of *pale* sherry, for one minute, rinse it off, and apply enough of A to cover the plate, for about the same time.

Now drop into the cup a drachm of B, and bring the A back from the plate to the cup to mix them together. Re-apply, and keep moving over the surface until density is sufficient. If any air-bells should occur they must be kept moving, and then they will do no harm.

Intensification can also be effected in a very marked manner by means of the following solutions :—

C.

Bromide of potassium...	17½ grains
Bichloride of mercury	17½ grains
Water	2 oz.

D.

Nitrate of silver	17½ grains
Cyanide of potassium	17½ grains
Water	2 oz.

C and D must be mixed separately and then mixed together, the plate being soaked in the solution until the necessary density has been obtained.

Both development and intensification are best performed in a dish—the former in an ebonite, the latter in one of porcelain. When the plate has again become dry, warm and varnish it as usual.

Fixing.—It is well to finish fixing each plate by immersing it for about ten minutes in a perfectly fresh hyposulphite solution after the plate has, in the ordinary sense of the word, been fixed—that is, after the creamy appearance of the film has been discharged by immersion in a solution perhaps not quite fresh. The temperature of the hyposulphite solution should not be below 50° nor above 70° F. The apparent change in the strength of the image which attends fixing has led some to suppose that the image is greatly weakened by the action of the hyposulphite solution; but this is not the case, and it is important to state clearly that the hyposulphite solution will not injure the image, even if it be allowed to act

for a very much longer time than is necessary for the thorough fixation of the image and the removal of unstable silver compounds from the film. Time must be allowed, both for the action of the hyposulphite of soda solution, and also for thorough washing afterwards, in order to effect the complete removal from the plate of compounds which, if not removed, will ultimately destroy the plate.

Finishing the Plate.—The plate must be dried spontaneously. On no account may artificial heat be applied. In case of urgency drying may be hastened by immersion in methylated spirit, and then varnished when dry. In every case the negative should be carefully preserved from damp. Enamel collodion is a useful temporary varnish for negatives on gelatine plates.

Having obtained a suitable negative, it is easy to print from this upon the ordinary sensitised paper, which may now be bought ready prepared at 1*s.* per sheet. It will keep in good condition for months.

After printing several shades darker than is finally required, the prints are soaked in water to remove the free nitrate of silver, and then put to tone in one of the following baths:—

I.

Chloride of gold	15 grains
Acetate of soda	1 oz.
Water	80 oz.

II.

Dissolve one 15-grain tube of chloride of gold in 2 oz. of water. Take 1 drachm of this solution, add 5 grains of tungstate of soda and pour in 3 oz. of boiling water. Use the bath ten or fifteen minutes after mixing; one grain of gold chloride will tone a sheet of paper.

III.

Chloride of gold	15 grains
Chloride of lime	15 grains
Precipitated chalk	1 teaspoonful
Water (boiling)	80 oz.

This may be used at once.

IV.

Chloride of gold	7½ grains
Perchloride of platinum	7½ grains
Acetate of soda	1 oz.
Water	80 oz.

After toning, the prints must be well washed and transferred to the fixing solution, which should be of the same strength as that used for fixing the negatives. The prints should be allowed to remain in this solution for ten minutes, after which they are transferred to water, and washed until perfectly freed from all traces of the hyposulphite.

There are two applications of this art which may be of great utility, viz., the preparation of wood-blocks for the purpose of illustrating microscopical work, and also for the production of lantern transparencies. The greatest expense in producing good illustrations is that of the artist, but if the picture is put upon the wood, the remainder of the work is cheaply and expeditiously performed. One way of doing this is to transfer an autotype carbon positive to the prepared wood-block; but to produce good pictures upon wood it is better to employ the wet collodion process.

A slightly over-developed positive is produced upon glass in the ordinary way, being developed with an iron developer, and the film transferred to the wood-block in the following manner. The block is first to be coated with a gelatine solution made by soaking 1 oz. of Nelson's gelatine in 19 oz. of water for twelve hours, dissolving with heat and stirring in a solution of 20 grains of chrome alum in 1 oz. of water. When thoroughly incorporated, add sufficient lamp-black to form an even black coating upon the wood.

If the block be first coated, it will be dry and ready to receive the film by the time the operator has proceeded thus far. The positive having been taken, developed, and

fixed, a piece of gummed paper is "squeegeed" to the wet film, which must then be stripped off the glass. The paper and film may now be trimmed with the scissors, and brought into contact under water with the prepared block, gently squeezed to remove air and water, and set aside for a while, so that the two films may become amalgamated. After a short time the block is soaked in tepid water to remove the gummed paper, and after drying is ready for the engraver. Many of the illustrations in this work have been prepared by this process, and it is here recommended to all who may read papers before the members of the various societies with which they may be connected.

In the preparation of transparencies for the lantern, it is necessary first of all to secure a good and sharp negative. This is placed above a dry plate, the two films being in contact, exposed to the light in a printing frame, and the latent image developed and fixed in the usual way. The best dry plates with which to prepare transparencies are the collodio-albumen, developed with pyrogallic and citric acids, and next to these, any good collodion plate. It is a very difficult matter to prepare *brilliant* transparencies upon gelatine plates; they are so extremely sensitive to light that it is very difficult to keep the high lights clear and free from fog. Very passable pictures may, however, be obtained from them by giving 12 seconds exposure, under a negative of ordinary density, to the light of a common bat's-wing gas-burner, developing the image with ferrous oxalate.

The collodio-albumen plates, when developed with pyrogallic and citric acids, generally yield a fine blue-black image; they may, however, be readily toned in a *weak* solution of sulphide of ammonium, if requisite.

When dry, the picture should be covered with another piece of clear glass the same size, the two being prevented from touching by very small strips of card fastened by gum

to two of the edges, the whole bound round with thin black paper, and the slide is completed. These pictures, when laid upon white paper, should only allow the paper to show through in the very highest lights.

For the demonstration of papers read before societies, transparencies prepared by the foregoing method may be shown by the use of the Sciopticon or Triplexicon, by which means sufficient illumination can be obtained with paraffin oil to produce a good disc 6 or 7 feet in diameter.

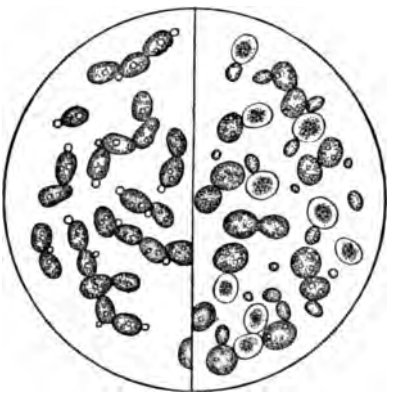


FIG. 254.

For the illustration of papers, very good results may be obtained by making drawings, by means of the ruled squares shown in Figs. 238 and 239, or by the help of the camera lucida. If these drawings are made twice the size (lineal) required and drawn with pen and ink, they may be reproduced by the process known as photo-zincography. For this purpose a good clear drawing, in which the shading is done by stippling, lines, or cross-hatching is necessary; and exceedingly good results are obtained by reducing the hand-drawn sketch to one half its size (lineal), as the lines are

thinned down by so doing, and the shading loses much of its harshness in the reduction. Many of the drawings have been prepared by this process for the present volume, notably Figs. 192, 220, 221, 247, 248, and 254, which last is a

FIG. 255.

FIG. 256.

drawing made from the microscope of a variety of the yeast plant.

Before closing this chapter, attention is called to the Figs. 255 and 256, to show how careful one must be in accepting the interpretations of nature as they come to us

through the engraver. Both these figures were cut from the same photograph, one being done in London, and the other in Manchester, the engravers being utterly unacquainted with microscopical subjects. Will the reader be able to recognise in the illustrations the spiracle of the *Dytiscus marginalis*, the great water beetle?

CHAPTER XIV.

MICROSCOPICAL MEASUREMENTS.

THE micrometer, as its name signifies, is used for measuring small objects, and omitting all historical notices—which may, however, be found in the works of Baker and Quekett—we will proceed to describe those forms most in use.

FIG. 257.

The stage micrometer consists of a glass slip such as is shown in Fig. 257, upon which is ruled a series of lines generally $\frac{1}{100}$ of an inch apart, one of these divisions being again divided into ten parts; so that there is on the same scale the means whereby objects of varying magnitude may be exactly measured, to less even than the thousandth of an inch.

For the unit of micrometry, the Committee of the American Society of Microscopists have adopted the *micron* ($\mu = \frac{1}{1000}$ *mm.*), and for their standard a centimetre scale, obtained for them by Professor Hilgard, the Superintendent of the U.S. Coast Survey. This scale was verified "with great care" by Professor C. S. Peirce in 1882, and again in 1883 by Professor W. A. Rogers. The scale was examined with a half-inch objective supplied with a Tolles' opaque illuminator, and the defining lines were found to be of the most beautiful

character. The scale is marked on a plate made of platinum and iridium (10 % iridium), and is divided into ten millimetres, one of the millimetres being divided into tenths, and one of the tenths into spaces of ten microns. The accuracy of the scale was determined by comparison with a standard which, it is stated, had been verified by Professor Tresca at Paris, and Mr. Chaney, the warden of the standards at London. In Europe the unit of measurement has been called a *micro-millimetre*, which is also the one-thousandth part of a millimetre.

The following table, extracted from the "Journal of the Royal Microscopical Society," may be of use to the reader :—

Fractions of an inch.	Micras.	Fractions of an inch.	Milli-metres.	Micras.	Decimals of an inch.
	μ		mm.	μ	in.
$\frac{1}{100000}$	2·539977	$\frac{1}{9000}$	·028222	1	·000039
$\frac{1}{90000}$	2·822197	$\frac{1}{8000}$	·031750	2	·000079
$\frac{1}{80000}$	3·174971	$\frac{1}{7000}$	·036285	3	·000118
$\frac{1}{70000}$	3·628539	$\frac{1}{6000}$	·042333	4	·000157
$\frac{1}{60000}$	4·233295	$\frac{1}{5000}$	·050800	5	·000197
$\frac{1}{50000}$	5·079954	$\frac{1}{4500}$	·056444	6	·000236
$\frac{1}{40000}$	6·349943	$\frac{1}{4000}$	·063499	7	·000276
$\frac{1}{30000}$	8·466591	$\frac{1}{3500}$	·072571	8	·000315
$\frac{1}{20000}$	12·699886	$\frac{1}{3000}$	·084666	9	·000354
$\frac{1}{10000}$	25·399772	$\frac{1}{2500}$	·101599	10	·000394
		$\frac{1}{2000}$	·126999		
		$\frac{1}{1500}$	·169332		
		$\frac{1}{1000}$	·253998		

The stage micrometer cannot be used for *direct* measurements with any save low powers, as it is necessary that the lines be seen at the same time as the dimensions of the object; but indirectly very exact measurements may be obtained. This may be accomplished by sketching the out-

lines of the object lying upon the stage, using the camera-lucida as shown in Fig. 241. The object is then to be replaced by the stage micrometer, the lines of which are drawn over the previous sketch, showing by simple inspection the magnitude of the object, or any part of it may now be measured with a pair of compasses.

It should be borne in mind that amplifications are generally expressed at a distance of 10 inches, and this is accomplished by placing the drawing slab horizontally 10 inches beneath the acute angle of the camera-lucida prism. If the object is required to be of the same size as it appears upon looking into the microscope, the distance of the drawing slab from the acute angle of the prism must be equal to the distance of that point from the objective. In estimating the magnifying power of an objective, the stage micrometer is employed and a sketch made of it upon the drawing slab at a distance of 10 inches. If, upon measuring these lines with an ordinary rule divided into inches and tenths, the hundredth of an inch space corresponds to one linear inch, it is clear that the object has been magnified 100 diameters; while if the thousandth of an inch space occupied half an inch on the rule, it would have been amplified 500 diameters.

Eye-piece micrometers are of several kinds, the simplest being a circle of glass ruled with a series of squares $\frac{1}{25}$ to $\frac{1}{100}$ of an inch (or $\frac{1}{10}$ of a millimetre) apart, being made to rest on the diaphragm of the eye-piece, or placed in front of the field lens in Ramsden's pattern. Previous to using this form of micrometer the value of the sides of these squares, with each objective, will have to be determined; this is done in the ollowing manner:—Place a stage-micrometer, ruled to hundredths and thousandths of an inch (or tenths and hundredths of a millimetre), in position upon the stage, and view it with the ruled circle *in situ*. The lines upon

both micrometers must now be rendered parallel, and carefully observed where the one overlaps the other, and if any uneven divisions appear, it will be better to pull out the draw-tube until they become even, keeping a record as to how much the tube has been withdrawn. As an instance—when using the 1-inch objective and an A eye-piece, each $\frac{1}{100}$ of an inch of the stage micrometer *nearly* coincided with ten divisions of the ruled glass circle. In order to make the coincidence exact, the draw-tube (Fig. 258) was pulled out $\frac{3}{10}$ of an inch; therefore, for that objective and ocular, each division of the eye-piece micrometer represents $\frac{1}{1000}$ of an inch, when the draw-tube is extended the same amount.

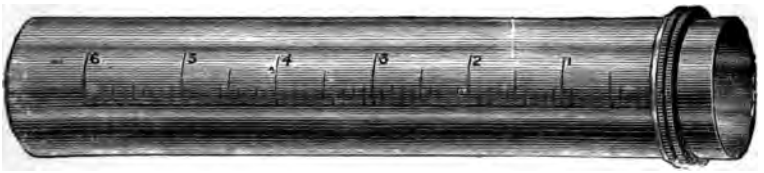


FIG. 258.

Jackson's eye-piece micrometer is shown in Figs. 259 and 260, inserted into the ordinary Huyghenian eye-piece. The micrometer itself consists of a series of lines ruled upon glass and set in a frame so that it may be moved forward by a small screw, a spring at the extreme end keeping it always in contact with it. The slits which are cut in each side of the eye-piece should be provided with a small sliding tube, to prevent dust entering the glasses.

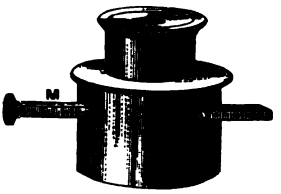


FIG. 259.

The method of finding the permanent values of the divisions of this micrometer is identical with that pre-

viously described, and when once found with accuracy should be entered in the observer's note-book for future reference.

Ramsden's screw micrometer, the last we need mention, is shown in Fig. 261. It is composed of a positive eye-piece (see page 93), before which is stretched two very fine parallel wires, one of which can be separated from the other by a

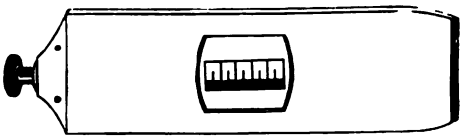


FIG. 260.

screw, having one hundred threads to the inch, the head being further graduated into one hundred parts, as shown in the figure representing the instrument made by Mr. Swift.

FIG. 261.

The field of view is illustrated by Fig. 262, and shows clearly the lower portion, consisting of a thin plate of brass, the edge being indented with a series of notches; every fifth indentation is deeper than the others, thus facilitating the

counting. Each notch is equivalent to one turn of the milled head, so that the number of revolutions may be read off without taking the eye from the object.

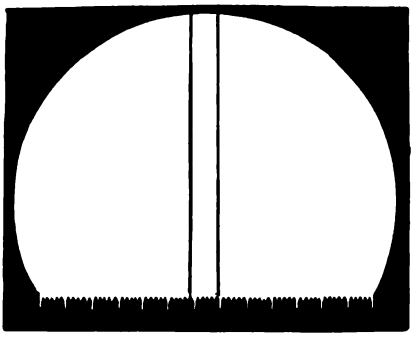


FIG. 262.

Quekett, in his "Practical Treatise on the Use of the Microscope," has shown the *theoretical* possibility of measuring a space so small as the eight-hundred-thousandth of an inch with the eighth objective and this micrometer; but

FIG. 263.

there are many practical difficulties in the way of attaining to this.

MEASUREMENT OF ANGULAR APERTURE.—Under the head of microscopic measurements may be described the

method of using Prof. Abbe's apertometer for measuring the aperture of objectives, an illustration of this instrument being shown in Fig. 263. It is a semi-circular disc of crown glass, 3·5 inches in diameter, and half an inch in thickness, polished on the cylindrical edge and ground to an angle of 45° upon the diametrical edge. The centre of the semicircle is formed by a small hole in a silvered cover *a*, cemented to the upper face of the disc. Two indices of blackened brass *b*, *b*, sliding on the periphery of the disc, afford visible marks for observing the aperture of any objective. On the upper face of the disc there are two engraved scales, the outer indicating the numerical aperture by direct observation.

In order to measure the aperture of any objective, the apertometer is placed upon the stage of a microscope, and the objective, the aperture of which is to be measured, roughly focussed to the small hole *a*, in the silvered cover. In the case of an immersion lens, a drop of immersion fluid must, of course, be applied. This done, the eye-piece of the microscope is removed and the eye placed to the open tube, the two indices of blackened brass *b*, *b*, being moved to and fro until the points just touch the margins of the illuminated field. The position of the indices is then read off on the outer circle of figures, and half the sum of both these readings will give the aperture of the objective.

For lenses of higher power than the ½-inch, a 2-inch objective is provided. This is made to screw into the lower end of the draw-tube and used with an ordinary A eye-piece, the whole being moved up and down until the edge of the disc is seen quite distinctly; the procedure is then the same as with low powers. This auxiliary objective must be furnished with a diaphragm to cut off all false rays, the aperture being one-eighth of an inch in diameter, and situated 1·5 inches from the achromatic combination.

The numerical aperture divided by the refractive index of the medium in which it is measured produces the sine of the semi-angle, and thus the angle in any medium may be easily obtained.

The following table of natural sines may be of use in this direction :—

NATURAL SINES.

Degrees.	Sines.	Degrees.	Sines.	Degrees.	Sines.
1	·0175	31	·5150	61	·8746
2	·0349	32	·5299	62	·8829
3	·0524	33	·5446	63	·8910
4	·0698	34	·5592	64	·8988
5	·0872	35	·5736	65	·9063
6	·1045	36	·5878	66	·9135
7	·1219	37	·6018	67	·9205
8	·1392	38	·6157	68	·9272
9	·1564	39	·6293	69	.9336
10	·1736	40	·6428	70	·9397
11	·1908	41	·6561	71	·9455
12	·2079	42	·6691	72	·9511
13	·2249	43	·6820	73	·9563
14	·2419	44	·6947	74	·9613
15	·2588	45	·7071	75	·9659
16	·2756	46	·7193	76	·9703
17	·2924	47	·7314	77	·9744
18	·3090	48	·7431	78	·9781
19	·3256	49	·7547	79	·9816
20	·3420	50	·7660	80	·9848
21	·3584	51	·7771	81	·9877
22	·3746	52	·7880	82	·9903
23	·3907	53	·7986	83	·9925
24	·4067	54	·8090	84	·9945
25	·4226	55	·8191	85	·9962
26	·4384	56	·8290	86	·9976
27	·4540	57	·8387	87	·9986
28	·4695	58	·8480	88	·9994
29	·4848	59	·8572	89	·9998
30	·5000	60	·8660	90	1·0000

Abbe's apertometer is one of the instruments which may be profitably purchased by the executive of every microscopical society, for use in testing the apertures of the members' objectives. Such a proceeding would often save the individual member much annoyance, and he would then be certain of

what he was purchasing. A case of this kind has just fallen under the author's notice. A $\frac{1}{8}$ objective was obtained of professedly 140° air angle, and £8 10s. paid for it, while when it came to be accurately measured, it turned out only 108°, and such a one could have been bought for 50s. It should be the duty of the executive of every microscopical society to secure its members against fraud, and the method above stated would most assuredly do this.

MEASURING EXACT FOCAL LENGTH OF OBJECTIVES.—The exact focal length of objectives may be easily arrived at by means of a formula described at some length by Mr. C. F. Cross, in the "Monthly Microscopical Journal," and on this account it is called Cross's formula. The measurements are better made by means of the photo-micrographic camera, shown at Figs. 244 or 245. For this purpose a micrometer ruled to $\frac{1}{100}$ths and $\frac{1}{1000}$ths is placed on the stage of the microscope, and an image of the lines projected upon the ground glass screen, where the distances are to be accurately measured, and from this the number of diameters to which the micrometer has been magnified can be easily determined. If now this amplification be indicated by n, and the distance between the micrometer and screen by l, the equivalent focus f of the lens may easily be calculated by the formula:—

$$f = \frac{n\,l}{(n+1)^2}$$

It is well to take l as a long distance, say 30 to 50 inches, as then slight errors of measurement are not of extreme importance. In three measurements of a reputed half-inch objective, the following numbers were obtained:—

	n	l	$\frac{n\,l}{(n+1)^2}$
a.	54	22·8	0·407
b.	78	32·5	0·406
c.	110	45·5	0·406

showing that accurate results may be obtained with only an ordinary amount of care.

MEASURING THE THICKNESS OF COVERING GLASS.—In the finer work appertaining to microscopy it is all-important to know the exact thickness of the cover-glasses used for mounting objects to be viewed with the higher powers. Many methods, no doubt, will have suggested themselves to the enthusiastic worker, such as that of noticing how many turns of the fine adjustment are required to separate the dust on the top side of the cover from that on the underside; but there are several instruments which can be used in a positive manner, and the thickness read off on a fixed scale. Of course, the reader will be familiar with the lever of contact

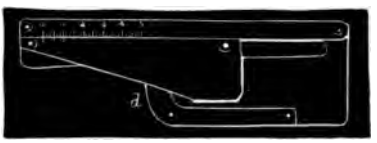


FIG. 264.

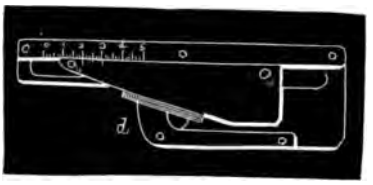


FIG. 265.

method used by Messrs. Ross and Co.; but there is no doubt that the measuring wedge devised by Herr Schönemann and illustrated by Figs. 264 and 265 is the more easily used. In order to measure the thickness of a covering glass, the instrument should be perfectly free from dust, and the wedge drawn back until the cover can be placed between the wedge and the piece *d*. The wedge is then closed until stopped by the glass, when the number of the divisions indicating the thick-

ness can be read off. A modification of this piece of apparatus was devised by Mr. H. Chaney, and is made by Messrs. Troughton and Sims.

No doubt the working microscopist will be called upon to make many other measurements in the course of his studies; but the foregoing will serve to show the nature of such operations, and the intelligent observer will always be able to devise means to effect the object in view.

CHAPTER XV.

THE POLARISCOPE.

WHEN a ray of light is made to pass in a certain direction through a crystal of Iceland spar, it becomes split up into two portions of equal intensity, an *ordinary* ray and an *extraordinary* ray respectively. If a ray, having undergone ordinary refraction through a crystal of Iceland spar, be allowed to pass through a second crystal, it is split up in the same manner; but the ordinary and extraordinary rays will be of different degrees of intensity. When the second crystal is rotated until the two are in similar or opposite positions, then the ordinary ray appears at its greatest intensity, while the extraordinary ray disappears. If the second crystal is further rotated the extraordinary ray reappears and increases in intensity as the rotation proceeds, while the ordinary ray diminishes in intensity until the principal planes of the crystals are at right angles to each other, when the ordinary ray vanishes and the extraordinary ray appears at its greatest intensity.

These and similar phenomena are called polarisation, and two prisms are required to render it patent—the first for polarising the light, called the "polariser," or the "polarising prism;" and the second, necessary for exhibiting

the fact that the light has undergone modification, called the "analysing prism," or simply the "analyser."

There are several methods of producing the polarisation of light, but the one usually employed for microscopical purposes is by the aid of the Nicol, or single image prism, which yields a beam perfectly colourless, polarising completely, and transmitting but one of the rays, the other being entirely suppressed.

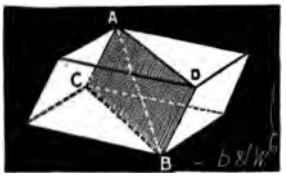


FIG. 266.

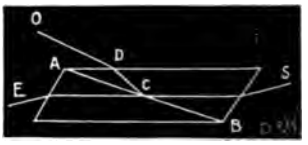


FIG. 267.

The Nicol prism is made from a rhombohedron of Iceland spar, bisected in the plane which passes through its obtuse angles, as shown in Fig. 266 by the letters A B C D, the halves being joined again in the same order by means of Canada balsam.

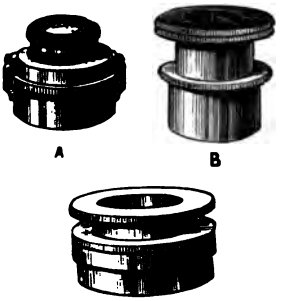


FIG. 268.

When a luminous ray enters this prism the ordinary ray undergoes total reflection, passing out of the crystal at O, while the extraordinary ray emerges at E, Fig. 267.

As attached to ordinary microscopes both polariser and analyser are formed of Nicol prisms; the former being made to fix underneath the stage or into the sub-stage. For this purpose it is mounted as shown in C (Fig. 268), so that the prism may be easily rotated by means of the milled head.

The analyser may be used in several ways. In the first, the Nicol prism is set in a mount screwing into the lower end of the microscope body, just above the objective, or in a brass box sliding into the side of the tube, in a similar manner to the Wenham prism of a binocular instrument. In another, the prism fits over the eye-piece in the place of the ordinary cap, each kind of apparatus being shown in Fig. 268, where the eye-piece analyser is shown at B, the other form at A. A section of the Prazmowski ocular analyser, as made by Zeiss, may be seen at Fig. 269.

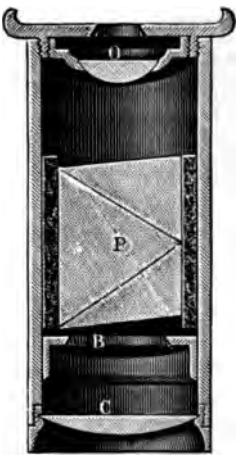


FIG. 269.

Sometimes a simple rhomb of Iceland spar, called also a "double image prism," is used as an analyser, but is not so generally useful as the Nicol prism previously described, though a series of interesting experiments may be made with it.

There are several other methods of polarising light: by means of the bundle of glass polariser, the plate of black glass, the tourmaline plate, and the sulphate of iodo-quinine crystal; but as all these can be seen in almost any treatise on optics, it seems scarcely necessary to more than mention them here.

The tourmaline plate may, perhaps, receive notice; in this crystal the optical axis coincides with the axis of the prism, and for optical purposes a plate is cut from it in a plane parallel to this axis. There are many objections to the use of a tourmaline plate, the principal being that a considerable thickness is required, as unless the ordinary ray is completely absorbed, the emergent light will be only partially polarised, and this requisite thickness produces a very undesirable colour.

When the analysing prism is placed immediately above the objective it stops a considerable amount of light, though it gives a full field, while when placed over the eye-piece a smaller field but brighter image is obtained.

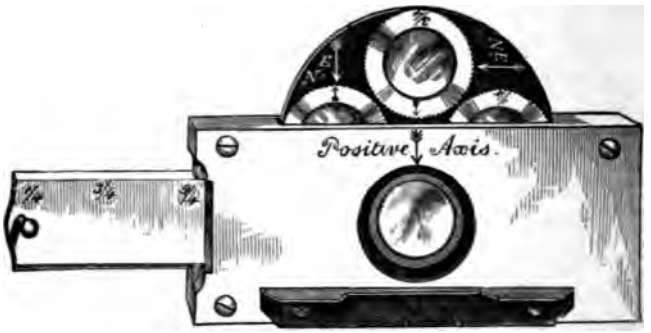


FIG. 270.

It is often useful to produce certain colour effects when working with polarised light, and with this end in view a plate of selenite is interposed between the polariser and the object. Films of varying thicknesses are used; they are

FIG. 271.

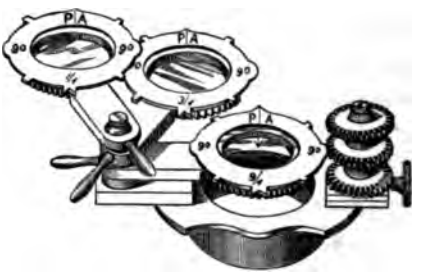


FIG. 272.

mounted in various ways, as selenite stages, object-carriers, and the like. Perhaps the best form of selenite stage is that made by Mr. Swift; it is shown in Fig. 270; the reader will be able to make out its construction from the illustration.

Mr. Swift's complete condenser, which has been already shown at Fig. 79, is also fitted with selenite discs for colour development, and also with a revolving film of mica, by the combinations of which a very great number of tints may be obtained.

Darker's selenite discs, as made by Messrs. Beck, are shown in Fig. 271, and are very useful, seeing that one or all of them can be put in and out of use in a moment. This form has been modified by Mr. Richards, who read a paper upon "A New Method of using Darker's Films" before the Royal Microscopical Society, March 9th, 1870. His improved apparatus may be seen in Fig. 272, having been reduced from the drawing which appeared in the "Monthly Microscopical Journal."

The real advantage of the apparatus (if any) seems to be in the application of gearing to produce revolution of the films, an operation easily performed by the fingers alone in the Ross instrument, as well as those of other makers.

The colours produced by the selenite films are brought about by variations in their thickness, the red being yielded by the thickest, violet by the thinnest; these films vary between ·00124 and ·01818 of an inch. The arrowhead and letters P A indicate the positive axis of the selenite, while the figures $\frac{1}{4}$, $\frac{3}{4}$, and $\frac{9}{4}$ denote the parts of a vibration retarded by each disc.

But the student need not go to much expense over these elaborate stages. They are intended to save time and not money, and the ordinary plain mounted selenite films on a 3×1 inch glass slide will answer many purposes, though incapable of rotation. If the red and green, blue and yellow slides be provided, with a film of mica, this is about all the student need purchase, at all events for some time to come.

Rock crystal or quartz possesses the extraordinary power

called rotatory polarisation. If a ray of homogeneous light be polarised, and the analyser turned so that it appears totally extinguished, the ray can be made to pass again through the analyser by placing a thin section of quartz, with its plane at right angles to the rays, between the polariser and analyser. In order to effect this the quartz crystal must be cut at right angles to its axis.

In most petrological microscopes of the present day Klein's plate of quartz is inserted in a movable box placed immediately above the objective. This plate is usually 3·75 millimetres in thickness, and is of the greatest utility when

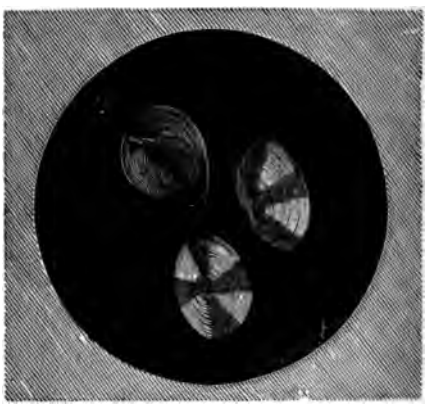


FIG. 273.

very feeble, doubly-refracting crystals are being examined, and in many other instances where it is suspected that isotropic particles exist in admixture with amorphous matter of a doubtful character.

The action of two Nicol prisms with an interposed plate of quartz upon a beam of light has been made use of for the purpose of registering the position of absorption bands in the spectra of various substances; but this properly belongs to the next chapter.

The development of the study of objects by the aid of

polarised light may, in the end, bring about important discoveries, and therefore should be studied more carefully than it is at present.

Let the student examine some potato starch by the use of ordinary light, and after noticing the position of the hilum, again examine with polarised light, when he will not fail to notice the black cross, as shown in Fig. 273.

For the observation of starches under polarised light, they should be mounted in gum dammar, dissolved in benzol—as in aqueous fluids the effect is not developed in so marked a manner. Wheat starch, which only faintly shows the cross

FIG. 274.

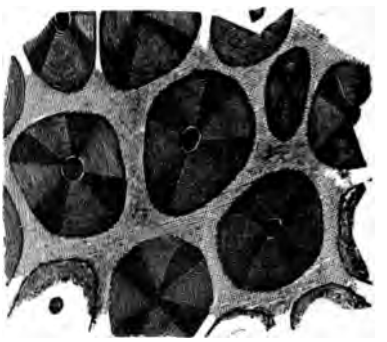


FIG. 275.

when observed in water, exhibits it very plainly in gum dammar and benzol. The effects of polarised light on the various starches may be plainly seen in tous-les-mois. The palates of molluscs are displayed remarkably well under the influence of polarised light, especially when mounted in gum dammar or Canada balsam. An illustration of the palate of the *Doris tuberculata* is shown in Fig. 274.

A very common object, seen in almost all collections of slides, is the section of rhinoceros horn. When examined by ordinary light it appears to resemble a bundle of hairs; but,

when viewed with the polariscope, each cylindrical aggregation is marked by a cross, shown by Fig. 275, bearing a striking resemblance to the starch granules, illustrated by Fig. 273.

The muscular system of many minute animals can only be satisfactorily made out by means of polarised light, which very strikingly differentiates the tissues; while such objects as silk, cotton, flax, and jute, are often to be distinguished from other substances by its aid.

But by far the most important application of the microscope and polariscope is in relation to petrology, whereby much regarding the nature of most minerals may be satisfactorily made out. With this end in view several petrological microscopes have been produced during the past few years, notably from the houses of Mr. Watson and Messrs. Swift and Son.

The instrument made by Mr. Swift is specially designed for the examination of rocks and mineral substances, being furnished with a concentric and revolving glass stage, self-centering arrangement, goniometer, and complete polariscope. The polarising prism is also provided with a divided circle and spring catch to indicate when the Nicols are crossed. It is made to slide up in its fitting close to the under side of the object, and on it are fitted condensing lenses of extreme high angle for showing the brushes in crystals. When not required, the polariser can be moved aside, entirely clear of the stage of the microscope. The analyser is made to slide in the optical tube, immediately under which, in the same way, is fitted a Klein's quartz plate. An extra analysing prism with divided circle is placed over the eye-piece, and a contrivance for rotating crystals between it and the prism is also added. The eye-piece has spider lines accurately adjusted to the plane of the prism.

When the Nicol prisms (polariser and analyser) are set at

right angles to each other, they are said to be crossed, and there is a total extinction of light passing to the eye of the observer; a thin section of an amorphous substance, such as glass, if placed upon the stage, does not produce any change, neither does a similar slice of any mineral which crystallises in the cubic system; and if the field remains perfectly dark when an object is observed between crossed "Nicols," it may be safely inferred that it belongs to one of these classes, or happens to be lying in a plane yielding single refraction only.

Some crystals appear not to polarise until the stage upon which the slide is placed be rotated, and if it becomes visible by this treatment it is certain the crystal does not belong to the cubic system.

Minerals of all the other systems of crystallography behave in various manners under crossed Nicols, and this branch of study may be easily carried out with the aid of the information to be found in the chapter devoted to section cutting. Sections should be cut in various planes of the crystal so as to represent the principal directions of vibration and also the axes of elasticity; but as this properly belongs to the science of petrology, the student should refer to those treatises in which these questions are specially considered.

In Swift's petrological microscope there is fitted an arrangement for showing the rings in biaxial crystals: a crystal of augite spar (*diopside*) is brought into the field of the microscope, and its entire system of rings may be exhibited as large, and with as much brilliancy, as in Norremberg's polariscope.

These coloured rings form a curious study; when, for instance, a section of Iceland spar (a negative uniaxial crystal) is cut from 1 to 20 millimetres thick in a plane perpendicular to the axis, and observed with polarised light, the following curious appearances are presented. First, when the axes of

both polariser and analyser are perpendicular, there is shown a beautiful series of coloured rings traversed by a black cross; and secondly, when the axes are parallel, the coloured rings possess complementary tints to those shown at first, while a coloured cross has taken the place of the black one.

In biaxial crystals the system of rings is more complicated, and this series may be well studied in a plate of nitrate of potash, cut perpendicularly to its axis.

In order to exhibit the phenomenon of double refraction, a "double-image prism" is used over the eye-piece, in conjunction with an objective; a plate of brass, pierced with a series of holes $\frac{1}{16}$ to $\frac{1}{4}$ of an inch in diameter, being laid upon the stage. When the polariser is fitted into its place below the stage, and the double-image prism over the eye-piece, there will appear (in some positions) two images, while, when the prism is moved, one of the images will be completely obliterated, a change taking place at every quarter of a revolution. When the polariser is taken away, the eye-piece shows two distinct images of the light circle, the ordinary and the extraordinary ray, which describe a circle upon revolving the eye-piece. If one of the larger apertures be now placed under the objective, the images will not be completely separated, but will overlap.

These are only a few of the many curious effects produced by polarised light, and its *study* should be more fostered and encouraged.

It is not enough to mount slides of salicine and other crystalline forms for the production of rainbow colours, and therefore the author imagines he cannot do better than give a list of the principal works which may be consulted:—"Lectures on Polarised Light," by T. P., Longman and Co., 1843; "Polarisation of Light," by William Spottiswoode, Nature Series, 1874; "A Familiar Introduction to the Study of Polarised Light," by C. Woodward, Van Voorst, 1861;

"Minéralogie Micrographique," MM. Fouqué and Lévy, published by the French Minister of Public Works, and is a most valuable work to all concerned in microscopic mineralogy.

To conclude this chapter, a list is given of some polarising objects, which may be studied by the beginner:—

ANIMAL SUBSTANCES.

Palate of limpet.	Human corns.
,, periwinkle.	Horn of rhinoceros.
,, whelk.	,, antelope.
Embryo oysters.	Skin of rhinoceros.
Human hair.	Alpaca wool.
Raw silk.	Porcupine quill.

VEGETABLE SUBSTANCES.

Starch from tous-les-mois.	Plant raphides.
,, potato.	Seed of Eccremocarpus.
,, arrowroot.	Cuticle of *Correa cardinalis*.
,, maize.	Fern scales.
Cotton fibres.	Jute fibres.

CHEMICAL CRYSTALS.

Salicine.	Asparagine.
Quinine.	Quinidine.
Chlorate of potash.	Strychnine.
Cane sugar.	Tartaric acid.
Brownian motion in fluids.	Citric acid.
Oxalic acid.	Sulphate of lime.
Sulphate of copper.	Chloride of barium.

CHAPTER XVI.

THE MICRO-SPECTROSCOPE.

THE micro-spectroscope is used as a means for exhibiting the absence of certain rays of light in a beam which has been modified by its passage through certain substances or solutions. Generally speaking, there are two patterns of the instrument, the former made by Messrs. Beck, as shown in Fig. 276, and that made by Mr. Browning, shown in Fig. 277. There is not much difference between the two in actual working, the form the author prefers, however, being that of Mr. Browning.

FIG. 276.

The Sorby-Browning instrument consists of a series of prisms, of crown glass and flint, mounted in a small tube, so that they can be removed at pleasure. Immediately below this tube is an achromatic eye-glass; and below the adjustable slit, placed about the centre

of the widened part of the tube, is an ordinary plano-convex field-lens.

The amount of dispersion, that is, the length of the visible spectrum, is produced by alterations in the prisms, and it is as well for the worker in this branch of microscopy to be provided with several copies of the tube, containing prisms yielding different degrees of dispersion. Mr. Browning has made for the author four such tubes, producing a small, medium, and large amount of dispersion, one of which can easily be substituted for the other.

FIG. 277.

The student must remember that an increased dispersion does not define the absorption bands any the better; on the contrary, fine and delicate lines, easily seen by the use of a prism with moderate dispersion, are often lost when one of large dispersion is substituted.

To use this instrument, insert it as an eye-piece in the

microscope, screw on the object-glass, and place the object, the spectrum of which is required, upon the stage. Illuminate by means of the sub-stage mirror if the object is transparent, or with mirror, Lieberkuhn, or side-reflector if opaque. Remove the tube A, and open the slit by means of the milled head H. Focus the object and then gradually close the slit till a good spectrum is obtained, and this may be further improved by throwing the object a little out of focus. Each portion of the spectrum differs a little from adjacent parts in refrangibility, and delicate bands or lines can only be brought out by focussing their own parts of the spectrum, which may be done by means of the milled head B.

One advantage which the micro-spectroscope possesses is that extremely small quantities may serve for observation, and for this purpose powers varying from $\frac{1}{3}$-inch to $\frac{1}{10}$-inch may be used.

FIG. 278.

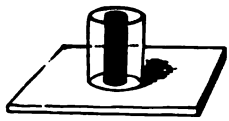


FIG. 279.

It is very easy to have the tints of the solutions examined too intense, so that nothing but a general and indefinite absorption appears throughout the entire spectrum. Nothing, perhaps, can show this better than a solution of permanganate of potash or Condy's pink fluid.

Mr. Sorby has devised what he has termed a wedge cell, in which to view the liquid for its absorption; it is shown in Fig. 278. By this means an increasing thickness of fluid and consequent intensity of colour can be brought under observation with the minimum of trouble. Again, very minute quantities of solutions may be examined in the slide shown in Fig. 279, made of a piece of barometer tube cut

and ground evenly, and then cemented to a piece of ordinary glass.

Mineral sections, crystals, blowpipe-beads, &c., should invariably have a small cardboard diaphragm, one-eighth diameter, placed beneath them; the spectrum is then much better defined. With a slide containing a mass of small crystals the object need merely be thrown a little out of focus.

When observing the spectra of liquids in experiment cells, or through small test-tubes, always slip over the 1½ or 2-inch objective a cap with a hole $\frac{1}{16}$ of an inch diameter. Slide this cap just sufficiently to bring the small hole a little within the focus of the objective. By this arrangement all extraneous light is prevented from passing up the body of the microscope, except what passes through the object. Unless this precaution be attended to, a false result is sometimes obtained.

Substances which give bands or lines in the red are best seen by gaslight, while those which give bands in the blue are brought out far better by daylight. Such a specimen as oxalate of chromium and soda is almost opaque by daylight, showing no bands, though when examined by a lamp the spectrum exhibits three beautifully fine lines in the red, two of which are exceedingly delicate. Again, uranic acetate can only be seen to advantage by strong daylight, since the band in the violet would be invisible by lamplight.

In solids, as crystallised uranic acetate, the tubes should be laid over a piece of black velvet on the stage of the microscope and illuminated with a bull's-eye condenser or side reflector.

It may be at first thought that absorption in the spectrum is due to the particular colour only—that is to say, a blue would always act as the ammoniacal sulphate of copper; a brown, as Parisite; a green, as chlorophyll; and all reds

would absorb the same light from the spectrum as a film of blood; but a few experiments would soon show that this is by no means the case, and a reference to Fig. 280 will show this clearly.

In the illustration, A represents the absorption spectrum of oxalate of didymium viewed by reflected light. The

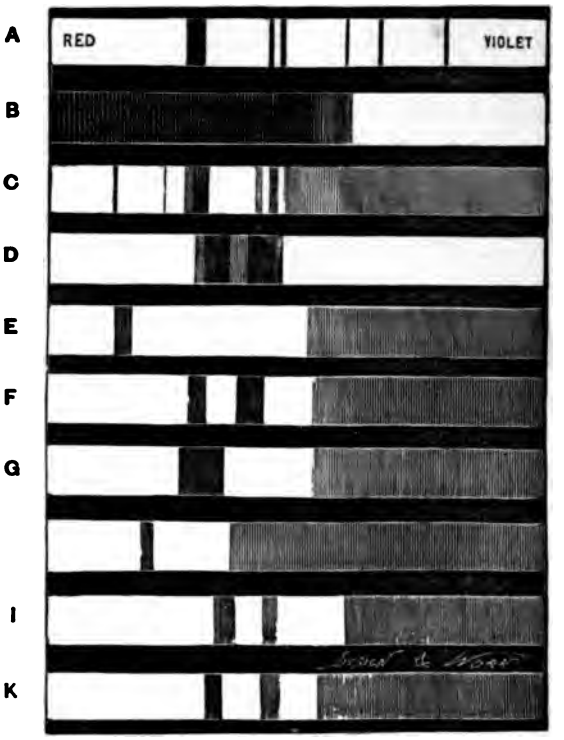


FIG. 280.

oxalate is a white powder, yet the six bands are very plainly defined even with ordinary instruments, while with a good set of prisms the broad band at the red end of the spectrum may be resolved into several dark lines.

The absorption bands of didymium were first described in 1858 by Dr. J. H. Gladstone, and are remarkable for their extreme sharpness.

At B is shown the spectrum of the deep blue solution of ammonio-sulphate of copper, already alluded to as being used for purposes of photo-micrography, and the figure here shows us how completely the red end of the spectrum is cut off, and how little of the actinic portion is absorbed. C is given to show how mixtures of substances may be detected; the illustration represents the absorption bands of Parisite—a brown and somewhat rare mineral from the emerald mines of New Granada. It consists principally of the carbonates of cerium, lanthanium, and didymium, the spectrum of the last showing out with its usual distinctness. At D may be seen the spectrum produced by a colouring matter obtained from the wing feathers of the Cape lory (*Turacus albocristatus*) by Professor Church, and to which he has given the name "turacine." E shows the spectrum (or one of them) of chlorophyll, and is characterised by the deep broad band in the red end of the spectrum; K, the orange colouring matter obtained from aphides by Mr. Sorby, remarkable on account of the band in the yellow. F, G, H, and I form a series; they are the spectra of blood and its modifications. F is fresh blood; G deoxidised blood; H blood treated with citric acid; while I is similar to H but deoxidised.

Mr. Sorby published an important paper on this subject in vol. vi. of the "Monthly Microscopical Journal." Fresh blood gives two dark bands in the green part of the spectrum, as is shown at F. This colouring matter is hæmoglobin, and the series of changes produced by several re-agents may be observed there. A small quantity of fresh blood diluted with a little water should be inserted in the tube shown in Fig. 279, and, examined upon the stage of the microscope, the two bands in the green will be

plainly perceived. Next add a trace of ammonia, a small quantity of Rochelle salt (double tartrate of potash and soda), and a particle of the protosulphate of iron and ammonia (ferrous amm. sulphate), and examine again. The two bands will gradually fade and be replaced by a fainter, though broad and single, band, as shown in G. If this be allowed to oxidise in the air, the two original bands of hæmoglobin can be seen again, and on adding a little citric acid the spectrum becomes changed to that seen in H. The citric acid has changed the hæmoglobin into hæmatin, and this latter can be further deoxidised by contact with ferrous sulphate, when it produces the spectrum shown at I.

Other spectroscopic changes can be induced in blood, but for these we must refer the reader to the tenth Report of the medical officer of the Privy Council, 1867.

In all work such as this it is advantageous to be able to throw up a second spectrum alongside the first for comparison; and in all good instruments this is provided for by fixing a small prism across half the slit, which receives its light from the *side* of the tube, dispersing it upwards to the eye.

For registering the position of the absorption bands two methods have been devised: (1) the quartz scale of Mr. Sorby, and (2) the bright spot micrometer of Mr. Browning. The first is constructed of two Nicol prisms, with an interposed plate of quartz, about ·043 of an inch in thickness, cut parallel to the principal axis of the crystal. This produces twelve interference lines or black bands in the spectrum, and is adjusted so that the sodium line of the solar spectrum is exactly midway between the third and fourth band, reckoning from the red end. This adjustment is performed by varying the thickness of the quartz plate, by no means an easy operation, as a very slight difference in thickness upsets the scale completely.

The Sorby quartz scale is small and convenient, and is shown at Fig. 281.

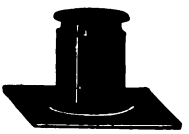


Fig. 281.

The use of this scale monopolises one of the two spectra, so that in many cases Mr. Browning's bright spot micrometer is decidedly preferable, as it does not preclude the use of either. The lateral piece AA is attached to the upper part of the prism tube, as shown in Fig. 282. At its outer extremity is an oblong box containing a glass plate, upon which is photographed a minute biconvex spot—bright on a dark ground. This is moved vertically by means of a screw connected with the graduated head M, the spot is illuminated by the small plane metallic mirror R, and its image formed by the lens C is capable of being adjusted for focus by the milled collar B. This image is reflected to the eye of the observer from the face of the upper prism, inclined at an angle of 45°, and is viewed along with the two spectra between which it appears to pass when the milled head M is turned. In the construction of maps, notice how many revolutions of the milled head M will carry the bright spot through the whole length of the spectrum, which will vary according to the dispersive power of the prisms.

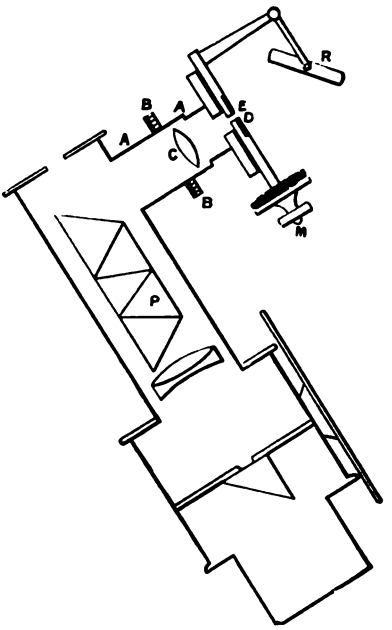


Fig. 282.

A centimetre will be found a convenient measure to represent one revolution: the millimetre will then equal ten of the smallest divisions of the milled head, or $\frac{1}{10}$ of a revolution; these smallest divisions, or thousandths, need not be regarded, as without them the spectrum will be divided with sufficient accuracy. Next, measure the distances between the Frauenhofer lines. This should be done with great care, as when once determined it will be constant. The lines A and *a* need not be measured, as they are in the dark part of the red, and are only to be seen in an extremely bright light, so will hardly be required in practice. The same remark will apply to the line H. The spectrum to be measured will therefore extend from about B to a little beyond G.

Let this scale be drawn on cardboard and preserved for reference. Now measure the position of the dark bands in any absorption spectra, taking care for this purpose to use lamplight, as daylight will give, of course, the Frauenhofer lines, and tend to confuse the spectrum. If the few lines occurring in most absorption spectra be now drawn to the same scale as the solar spectrum, on placing the scales side by side, a glance will show the exact position of the bands in the spectrum relatively to the Frauenhofer lines, which, thus treated, form a natural and unchangeable scale. But for purposes of comparison it will be found sufficient to compare the two lists of numbers representing the micrometric measures; simply exchanging copies of the scale of Frauenhofer lines, or the numbers representing them, will enable observers at a distance from each other to compare their results, or even to work simultaneously on the same subject.

Owing to the sliding and other fittings between the prisms and the slit, the micrometer cannot be depended on for pointing to the position of a line by setting it to the number recorded on the scale, but is only to be used for measuring between lines, and for this purpose it may be trusted.

Dr. Lawson has suggested that the great advantage of this contrivance is, that it does not monopolise one of the two spectra, as with the use of the quartz scale; for in describing two spectra, only slightly differing from each other, it may be used at once to determine the difference between them.

In concluding this chapter it may be as well to give a short list of substances showing well-defined absorption bands, such as may be of use to the student upon commencing to use the instrument.

Nitrate of didymium.
Oxalate of ,,
Oxide of ,, (blowpipe-bead).
Chloride of cobalt (in alcohol).
,, ,, (in chloride of calcium).
,, ,, (a crystal).
Cobalt glass (blue).
Sulphate of uranium.
Acetate of ,,
Oxalate of chromium and soda.
Sulphate of chromium.
Permanganate of potash.
Ruby glass (copper).
Ammonio-sulphate of copper.
The aniline colours.
The naphthalene colours.
Indigo sulphate.
Carmine.
Litmus (blue and red).
The colouring matters of plant petals.

CHAPTER XVII.

STAINING AND INJECTING.

In many instances it is necessary to stain a preparation, *not merely to form a pretty object*—let this be understood at the outset—but to show certain details of formation not easily discerned in an unstained specimen. When aniline blue and magenta are used for the double staining of vegetable tissues, such as the section of a Burdock stem, Mr. Barrett tells us the different parts are stained as follows:—

Pith	Very pale magenta.
Cellular tissue	Deep magenta.
Spiral vessels of medullary sheath	Deep blue.
Pitted vessels	Blue.
Cambium	Deep blue.
Liber cells	Dark magenta.
Laticiferous vessels	Deep blue.
Cuticle parenchyma	Pale blue.
Epidermis	Deep blue.
Hairs	Pale magenta.

Stems of all kinds should, if possible, be cut when fresh, when required for staining. If they cannot be obtained in this state, they may, previous to cutting, be soaked in cold or tepid water, or in a mixture of equal volumes of spirit of wine, glycerine, and water. Fresh stems or roots can be

preserved in this medium for almost any length of time, and will remain in excellent condition for the section machine.

LOGWOOD STAINING.—Wood sections require bleaching before being stained. The bleaching solution is made by mixing ¼ oz. of chloride of lime with a pint of water, shaking occasionally for an hour, and after allowing the sediment to subside, decanting the clear solution. The process of bleaching should be carefully watched and stopped when complete. Tissues vary so much in colour and density that no fixed time can be given for bleaching them. Very thorough washing is necessary. The elimination of the chlorine will be much facilitated by placing the sections, after removal from the bleaching liquid, in a solution of sulphite of soda (1 drachm to 4 oz. of water) for an hour, then washing the sections by soaking them for at least six or eight hours in water, changing occasionally, and finishing with distilled water. If they are not to be stained at once, they should be preserved in water containing 20 per cent. of alcohol, as, when kept in water only, in the course of two or three days they become covered with a peculiar fungus growth. At this stage all air-bubbles should be removed from the tissue. This is conveniently done by placing the sections in dilute alcohol, putting them under the receiver of an air-pump, Fig. 283, and exhausting the latter, repeating the pumping occasionally so long as air-bubbles are given off. For this purpose a small tube bottle is employed about 1½ inch long, and a receiver just large enough to hold it, the process being thus rendered a rapid one.

Where it is required to uniformly stain the section in order to render prominent the more delicate cell walls, logwood answers exceedingly well and is very permanent.

LOGWOOD SOLUTION.

Logwood, in coarse powder	2 oz.
Distilled water	10 oz.

Boil for half an hour in a glass beaker, replacing what is lost by evaporation; strain, and to each ounce of liquid when cold add 60 grains of alum and one drachm of alcohol, rub well together, filter through paper, and preserve in a stoppered bottle.

Staining Process.—Make a filtering cone by twice folding a piece of filtering paper about 1½ inch diameter; support this in the neck of a small beaker or tube, and

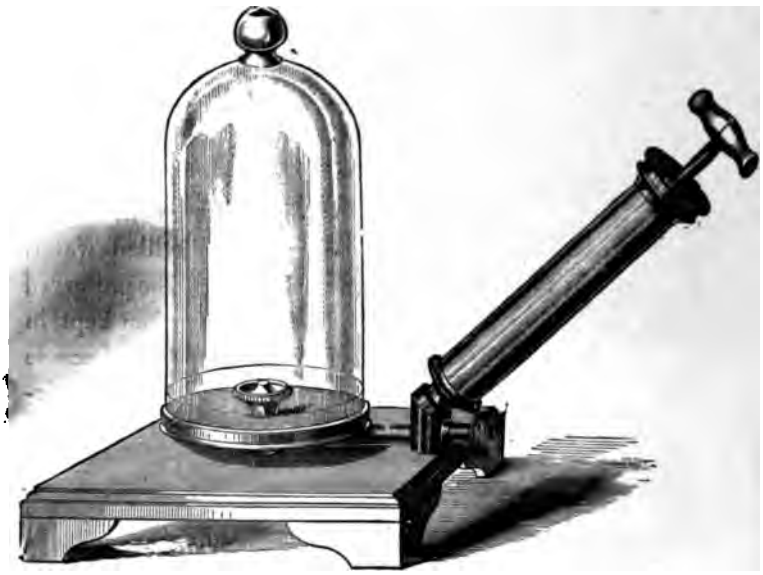


FIG. 283.

filter through it about ten drops of the above liquid, add thirty drops of distilled water, place the sections in the mixture for five minutes (more or less), pour off the stain, wash once or twice in distilled water, then soak for half an hour in a solution of alum (20 grains to the ounce), remove this, wash well with distilled water, and preserve in alcohol so as to be ready for mounting.

The logwood solution prepared in this way gives more

satisfactory results than when made as usually recommended from extract of logwood, the latter being a very variable article.

For double staining, which is more generally useful than the method just described, many formulæ have been given, the process having originated with Mr. G. D. Beatty, of Baltimore, U. S. A.

Perhaps the best paper in this country upon the subject is that which appeared in the pages of "Science Gossip," for January, 1880; but even the process therein described is in several points open to modification and improvement.

STAINING IN CARMINE AND GREEN.—The art of staining in carmine and green consists of five stages or processes—1. Decolorising the sections; 2. Washing the same; 3. Preparing for staining; 4. Staining in carmine; 5. Staining in green.

After bleaching and thoroughly washing to eliminate all the chemicals, as already described, in order to obtain deep colours, the sections must be steeped in a mordant composed of a 10 per cent. solution of alum and water for twenty-four hours, at the end of which time they will be ready to be placed in the first staining fluid, the formula for which is as follows:—

Carmine	15 grains.
Ammonia	15 grains.
Water	2 oz.

The carmine is to be dissolved in the ammonia over the flame of a spirit-lamp, the water added next, and the fluid filtered before it is used.

Immerse the sections in this stain for six or eight hours, then take them out, and wash them in not more than two changes of water, and finally transfer them to the green stain, for which take—

Aniline green	5 grains.
Absolute alcohol	1 oz.

Dissolve in a test-tube, using a slight heat only, to avoid any unpleasant mishap, and filter before using. After a three hours' soaking in the above, the staining will be completed, and the sections should be mounted without delay, after having washed the superfluous colour away with methylated spirit. The method of mounting these will be given later on.

STAINING IN PICRO-CARMINE.—Picro-carmine is the most truly selective of any double stain yet employed. A special modification of it is required for wood sections as follows:—

PICRO-CARMINE SOLUTION.

Carmine (finest)	2 grains.
Liquid ammonia (sp. gr. ·960) ...	½ drachm.
Distilled water	1 oz.

Put the carmine in a 2-ounce stoppered bottle, pour in the liquid ammonia, and shake occasionally until dissolved, then add the water.

Picric acid	8 grains.
Alcohol	1 oz.

Dissolve in a test-tube with a gentle heat, then mix with the solution of carmine.

Staining Process.—Place the sections in 50 per cent. alcohol for one hour, then treat with the recently filtered staining solution until the desired effect is produced (usually from half to two or three hours), remove the dye, wash quickly three or four times with alcohol 50 per cent., then soak in an alcoholic solution of picrate of ammonia, changing this at the expiration of an hour, and allowing the sections to remain in the second solution for about the same period.

When stained with logwood the sections, after being well washed, are soaked in alcohol for an hour, then removed to

oil of cajeput, and allowed to remain in this for a couple of hours. At the end of this time transfer them to oil of turpentine. In less than an hour they will be ready for mounting in balsam or dammar. The sections should not be allowed to remain long in the turpentine or else they become brittle.

In the case of picro-carmine stained sections they should be removed from the alcoholic solution of picrate of ammonia into alcohol for about a minute, then into oil of cajeput.

The object of employing an alcoholic solution of picrate of ammonia is to avoid the loss of colour which attends the use of alcohol only, the yellow stain of picric acid being readily removed from the tissue by that liquid. Picrate of ammonia may be easily made by adding a slight excess of liquid ammonia to a solution of picric acid, and evaporating the mixture to dryness at a gentle heat.* The residue is dissolved in alcohol and filtered.

Wood sections stained in picro-carmine are very beautiful and permanent. The staining being done at *one* operation, and the colours being remarkably selective, there is an absence of secondary tints, as in the case of most other double stains, especially where one tint is partially washed out to make way for another.

Regarding permanence, some stained sections mounted nearly ten years ago appear to have retained their brilliancy unimpaired.

In place of alcohol, methylated spirit may be used if desired.

The chromo-lithograph, forming the frontispiece to this work, illustrates very clearly the value of the foregoing methods. The four outside figures are specimens of double-staining in carmine and green, while the centre figure is a

* This salt had better be purchased by the student, as the picrates explode violently when over-heated.

representation of the slide sent by Mr. Stiles, of Doncaster, to illustrate his paper on picro-carmine staining in "The Northern Microscopist."

Histological (animal) staining differs somewhat from the foregoing, many processes having been devised to strikingly differentiate the tissues. In 1876, Dr. Elizabeth Hoggan described a process with iron and pyrogallic acid as follows:—

"The colouring agents required are a one or two per cent. solution of perchloride of iron in alcohol, and an alcoholic solution of pyrogallic acid of similar strength."

The section or membrane to be stained is first treated for a short time with alcohol, the iron solution filtered upon it, and then poured off after a couple of minutes. The pyrogallic solution is then filtered upon it, and when the desired depth of staining has been obtained, the tissue is washed and mounted in any of the usual ways.

The nuclei and nucleoli are by this means coloured black, and the cell-substance coloured more or less. A bluish tint may be imparted by washing in alkaline water or in water highly charged with carbonate of lime.

Osmic acid has been frequently recommended for staining animal tissues black, but it is doubtful whether anything is gained by its use. Dr. G. Brösicke, of Berlin, advises a mixture of osmic and oxalic acids for this purpose. Thin sections of animal tissues are placed in a one per cent. osmic acid solution, and then carefully washed to remove all superfluous acid. They must then be immersed for about twenty-four hours in a solution of one part of oxalic acid to fifteen of water, when, after washing, they are ready for examination.

The oxalic acid produces darker or lighter shades (carmine and Burgundy tints) in proportion to the length of time the section has been immersed in osmic acid, but if the tissue

has once become blackened, the oxalic solution is powerless to redden it afterwards.

Chloride of gold solution has been employed for colouring nerve-fibres; a solution in water of one or two grains to the ounce is generally used. The section should be soaked in the solution until it has acquired a straw-coloured tinge; it is then to be washed and placed in a one per cent. solution of acetic acid. In the light the nerve-fibres become coloured a blue or violet tinge in a few hours. Nitrate of silver is a very important agent for the staining of animal tissues. Dr. Lionel Beale writes, "A weak solution may be imbibed by

FIG. 284.

delicate tubes, and part being precipitated in the tube, perhaps as a chloride, or in combination with some albuminous material, subsequently becomes decomposed by the action of light."

Picro-carmine is also a good staining material for animal sections. Fig. 284 is a representation of a portion of the muscle of a man who died from trichinosis at Chorlton workhouse. Before staining, the trichinæ were not very visible, but after staining with picro-carmine they were easily seen.

Recklinghausen, who has employed this plan with much success, uses a very dilute solution, made by dissolving one grain of nitrate of silver in from one to two ounces of distilled water.

In 1863, Dr. Roberts, of Manchester, used a solution of tannin and magenta for staining the red human blood-corpuscles, and this is a subject which still requires investigation.

Dr. Beale's carmine fluid for staining all forms of *bioplasm* of living things is made as follows:—

Carmine	10 grains.
Liquor ammoniæ	½ drachm.
Glycerine	2 oz.
Distilled water	2 oz.
Alcohol	½ oz.

The carmine is to be dissolved in the ammonia, boiled for a few seconds, and set aside for an hour; the glycerine, water, and alcohol may then be added, and the whole allowed to stand until thoroughly settled, when the clear fluid is to be decanted and kept for use. Dr. Beale tells us that if the solution be very alkaline, the colouring will be too intense, and much of the soft tissue round the bioplasm will be destroyed by the action of the ammonia; if, on the other hand, the solution is neutral, the uniform staining of tissue and bioplasm may result. In some cases the fluid must be diluted with alcohol, water, or glycerine, in order to get the best results; and Dr. Beale observes that the process should not be hastily condemned without trying the effect of modifying the quantities of the various constituents.

There are many other processes to be found scattered through the pages of microscopical literature, but want of space compels us to close the subject here.

INJECTING.—The process of injecting should now claim our attention, though it is feared that actual practice with the

syringe will be found of far more use than any written instructions can ever be.

The term injecting, in its micrological application, signifies the filling of the arteries, veins, and other vessels of animals with coloured substances for the purpose of showing their arrangement in, and their course through, the tissues.

Proficiency in the art of making anatomical injections can be acquired only by continued practice, and by the exercise of patience and perseverance. The same remark applies even more strongly to the injecting of diseased tissues which have been excised from the living body, as in this case there are so many vessels, which have been severed, requiring to be ligatured to prevent the escape of the injection. The beginner is recommended to note carefully the causes of each failure, and to take precautions to avoid these in his subsequent practice. If this is done, the art of injecting will be learned sooner and more easily than it otherwise could be.

There are three well-known methods of making injections, which may be distinguished as follows:—

(1) Injections made with a syringe;

(2) Mechanical injections, in which the function of the syringe is replaced by the pressure of a column of water or mercury;

(3) Natural injections; or the introduction of pigments into the circulation of living animals.

The natural method is generally resorted to in cases where the two preceding processes are either altogether impracticable or very difficult to perform; as, for example, the filling of the bile ducts throughout their course in the liver.

Injecting by the pressure apparatus is very convenient when time cannot be spared to do the work by hand; but the latter method is *the one* which should be mastered, on

account of its simplicity when once learned, and the ease with which it can be performed.

The substances used for making injections may be divided into two classes: one class includes all those which are fluid at the ordinary temperature; while the other class includes such as become fluid only when heated, and return again to the solid form on cooling; these are called "masses."

The following are the most useful injections:—

Prussian Blue Fluid:—

Glycerine	1 oz.
Methylated Spirit	1 oz.
Ferrocyanide of Potassium	12 grains.
Tinct. Perchloride of Iron...	1 drachm.
Water	4 oz.

Mix together the glycerine, spirit, and water, and divide the mixture into two equal parts. In one part, dissolve the Ferrocyanide of Potassium (*a*), and to the other part add the Tincture of Perchloride of Iron (*b*); *b* must now be added very gradually to *a*, the mixture being well shaken after each addition of the iron solution. Keep this fluid in a stoppered bottle, and shake it well before using it.

Turnbull's Blue.—Dr. Beale, in his work on the microscope, says: "My friend, Mr. B. Wills Richardson, of Dublin, has introduced Turnbull's Blue in preference to ordinary Prussian Blue. Ten grains of pure Sulphate of Iron are to be dissolved in an ounce of glycerine, or better still, in a little distilled water, and then mixed with glycerine, and thirty-two grains of Ferridcyanide of Potassium in another small proportion of water and the solution mixed with glycerine. These two solutions are then gradually mixed together in a bottle, the iron solution being added to that of the Ferridcyanide, and the mixture ensured by frequent agitation." The deep blue fluid thus prepared must be added to one ounce of glycerine, one ounce of methylated spirit and four

ounces of water, as in the Prussian Blue fluid. Dr. Beale considers these proportions unnecessarily large, and gives the following recipe, which, however, has not been found to answer well, the injection, especially under high powers, being too faint.

Ferridcyanide of Potassium	10 grains.
Sulphate of Iron	5 grains.
Water	1 oz.
Glycerine	2 oz.
Alcohol	1 drachm.

The advantage of this injection over Prussian Blue is that the colour does not fade in course of time.

Brücke's soluble Prussian Blue.—

A. Ferrocyanide of Potassium, 217 grammes dissolved in one litre of distilled water.

B. Perchloride of Iron, 10 grammes dissolved in two litres of distilled water.

C. A cold saturated solution of Sulphate of Soda.

Mix one part of A with one part of C (α), and mix one part of B with one part of C (β). Add β to α, and allow the mixture to stand about three hours (longer if necessary), collect the deposit on a filter. Wash the deposit three or four times a day for a week by pouring over it small quantities of distilled water. The washing must be discontinued as soon as the water which runs through is quite blue; and the powder thus prepared must be dissolved in distilled water and mixed with sufficient gelatine to form a firm jelly.

Dr. Beale's Acid Carmine Fluid.—Dr. Beale says, "After trying a great many different combinations, I arrived at the following, which answers the purpose exceedingly well:—

Carmine...	5 grains.
Glycerine, with 8 or 10 drops of Acetic or Hydrochloric Acid ...	½ oz.
Glycerine	1 oz.
Alcohol	2 drachms.
Water	6 drachms.
Ammonia	a few drops.

Mix the carmine with a few drops of water, and, when well incorporated, add about five drops of liquor ammoniæ. To this dark red solution about half an ounce of the glycerine is to be added, and the whole well shaken in a bottle. Next, very gradually pour in the acid glycerine, frequently shaking the bottle during admixture. Test the mixture with blue litmus paper, and if not of a very decidedly acid reaction, a few drops more acid must be added to the remainder of the glycerine, and mixed as before. Lastly, mix the alcohol and water very gradually, shaking the bottle thoroughly after each successive portion till the whole is mixed. This fluid, like the Prussian Blue, may be kept ready prepared, and injections made very rapidly with it." This is, without doubt, one of the best fluid injections ever devised. It is particularly useful for injecting such lower forms of animal life as insects, shell-fish, snails, and small fishes.

Acetic acid is to be preferred to hydrochloric for the purpose of acidifying the solution. The object in adding acid to carmine injections is to precipitate the carmine, and so prevent it from transuding through the walls of the vessels into which it is thrown.

Dr. Carter's Carmine.

Carmine	60 grains.
Liq. Ammonia (B. P.)	180 minims.
Glacial Acetic Acid	86 minims.
Gelatine (Gel. 1 part, water 6 parts) ...	2 oz.
Water	4 oz.

The Carmine is to be dissolved in the ammonia and added to the water. This solution is added to one half of the gelatine, and to the remaining half of gelatine is added the acetic acid. The acidified gelatine solution is next mixed drop by drop with the portion containing the carmine, and the whole is filtered through fine flannel before use. To be successful in making this mass, it is necessary to use *glacial* acetic acid, and the *strong* liquor ammonia of the British Pharmacopœia.

A good supply of each of these injections should be kept ready for immediate use. It is convenient to keep the masses in vessels made either of block tin or copper, in order that they may be readily heated. They may, however, be kept in earthenware jars and melted by placing the jars in boiling water. The fluid injections should be kept in stoppered bottles, and the mouth should be sufficiently large to admit the nozzle of the syringe. The different injections should be filtered occasionally to remove any particles of matter which may get into them, and they should be distinctly labelled; this precaution will effect a saving in time and prevent mistakes.

The Syringe.—In selecting a syringe, the following points should be attended to. (1) The syringe, which should be of at least one ounce capacity, should be furnished with two rings at its upper end, one on each side, for the fingers to pass through; (2) It should also be furnished with three pipes or canulæ of about $\frac{1}{16}$ in., $\frac{1}{32}$ in., and $\frac{1}{64}$ in. diameter; and, in order that they may be secured firmly in the vessels whilst making an injection, they should be provided with a pair of arms to pass the ligature round; (3) The piston should fit the cylinder so accurately that if the nozzle of the syringe be closed with the finger, and the piston be drawn up, it will, on being released, instantly return to its former position; (4) The syringe should be provided with a stopcock. A syringe of this kind costs about 15*s.* If the beginner does not desire to go to so much expense, a glass syringe, costing about 1*s.*, may be used. The canulæ can be made out of glass tubing, by drawing it to a fine point in a Bunsen's flame, and then cutting off the part required.

Destroying the Life of an Animal intended for Injection.—The life of an animal intended for injection is destroyed most easily and in the best manner by opening it from anus to throat, and cutting deeply into the heart across the right auricle. This

is, of course, done whilst the animal is under the influence of chloroform, or even immediately after it has been suffocated by chloroform. To facilitate the bleeding, the animal should be suspended alternately by the hind and front legs, and as the blood coagulates in the wound in the heart, it should be removed. The best way to administer the chloroform is to place the animal in a box, drop in a piece of cotton wool, saturated with chloroform, and close the lid. In from five to fifteen minutes the animal will be dead. Half an ounce of chloroform is quite sufficient to kill any cat, and the same quantity should suffice for a dog a foot high.

Injecting a whole Animal.—A young animal is best for this purpose. A rabbit is, perhaps, the best subject for a beginner to select. After having killed it, immerse the body in hot water for about fifteen minutes; then take it out, pass a ligature round the aorta close to the heart, make a longitudinal incision in the aorta, and insert the canula of most suitable size. Bind the canula firmly in the artery, and attach the stopcock. Oiled worsted is the best substance which can be employed for tying the pipes in the vessels; it should not be drawn too tight or it will cut through them, and so permit the pipe to come out. *All* vessels must be opened longitudinally, *not* transversely, or they would probably contract so much as to exclude the possibility of making an injection.

A good supply of hot carmine mass (Dr. Carter's Carmine) should be ready for use. Fill first the syringe with the injection, and then the stopcock and canula. Then insert the nozzle of the syringe into the stopcock, taking care that in doing this no air is admitted, or it will be forced into the vessels, and the passage of the fluid impeded. These points having been attended to, the injection should commence. The amount of pressure exerted on the piston should at first be very slight; but it will be necessary to increase it as the

injection proceeds. It is advisable to support the animal in the water either with the left hand, or to allow it to rest at the bottom of the vessel containing the water. The filling of the spleen should be watched carefully, and as soon as it is fully distended, more injection mass should be prevented from flowing into it by tying a ligature round its artery. The splenic artery is easily found. It arises as a branch of the cœliac axis, and enters the substance of the spleen at the hilus on its concave surface. In order to obtain a perfect injection of the kidney, it should be drained of all blood by opening the renal vein. Blood and carmine mass will at first flow out together; but, as soon as the carmine flows out freely and unmixed with blood, the vein should be ligatured, and the vessels allowed to fill slowly. The injection may be considered complete when the transparent parts about both the upper and lower extremities show a reddened and slightly distended appearance. The internal organs, when well injected, have a deep red colour, and appear as if inflated with air. In this operation, the lungs remain untouched by the injection, and they must therefore be injected separately through the pulmonary artery, either *in situ*, or after they have been excised. In order to render the capillaries of the alveoli perfectly distinct in section, it is usual to distend the air cells of the lungs by pouring melted cocoa-nut oil down the trachea. The oil, on cooling, solidifies, and makes the cutting of extremely thin sections after hardening an easy matter. After the injection is completed, the open vessels should be tied, and the animal placed in cold water; half an hour afterwards, the different parts should be dissected out, and placed in methylated spirit.

In passing, it may be remarked that some histologists consider it preferable to inject the entire animal, not through the aorta, but through the carotid arteries; this operation is, however, much more difficult than the preceding one, and

there is no real necessity for proceeding in this way when the former method yields excellent results.

Hardening Injected Tissues.—Injected tissues must be hardened in spirit. After the first day's immersion they should be transferred to fresh spirit for two more days, and then into fresh spirit again, and kept in this until ready for cutting into sections. It is *never* necessary to use absolute alcohol as a hardening agent, and it is seldom needful to place the tissues first in weak spirit and gradually to increase the strength up to perfectly anhydrous alcohol. The length of time required for hardening depends upon the kind of tissue, its size, and, to a certain extent, upon the quantity of spirit used; the smaller the size of the tissue, the more rapidly will the hardening be done. Brain, kidney, and spinal cord are rendered sufficiently hard for cutting into sections in three weeks; lung, liver, spleen, pancreas, the intestines, the tongue, etc., take a longer time, usually from five to eight weeks. A saturated aqueous solution of picric acid is sometimes used as a hardening agent; but its action as a very persistent yellow dye is much against its employment for this purpose.

Injecting Separate Parts.—Having now described the method of injecting an entire animal, we will pass on to an account of the modes of injecting different organs and parts of an animal.

The Kidney.—On account of the comparatively large size of the blood-vessels of the kidney, it is a very suitable organ for the beginner to practise upon. The filling of the arteries should first be mastered, and afterwards the injecting of both the arteries and veins. To inject the arteries, tie the canula in the renal artery, and throw in carmine mass or Prussian blue. When the vessels are about half filled, the injection will begin to flow from the renal vein, which should then be ligatured, and the vessels slowly filled. After this is

done, place the organ in spirit or cold water to cause the jelly to set, and then cut it into pieces of a suitable size for hardening. A good preparation will show the medullary portion filled with long and slightly curved arteries running parallel to each other; the artery or afferent vessel entering the Malpighian tuft, the vein or efferent vessel leaving it, and the whole of the vascular portion of the kidney surrounding the Malpighian tufts filled with a network of arteries, capillaries, and veins. If a double injection is required, inject the vein first with Prussian blue or Turnbull's blue, and afterwards the artery.

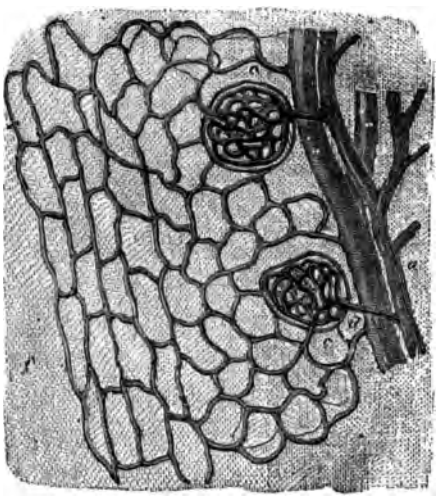


FIG. 285.

Fig. 285 shows a longitudinal section of the injected kidney of a rat, *a* being the arterial trunk, *b* the venous trunk, and *c* the glomerulus, or Malpighian tuft, an extremely pretty and instructive preparation when properly prepared.

The Liver.—If the entire animal has been injected from

the aorta, only the vessels supplied by the hepatic artery will be filled. In this case, the portal vein should be injected with one colour, and the hepatic vein with another.

The method of injecting the bile ducts in a satisfactory manner has long puzzled anatomists before Chrzonszczewsky introduced his method of natural injection. The animal having been chloroformed, a solution of indigo-carmine is to be introduced into the jugular vein. The dose should be repeated several times, and the animal killed in about an hour and a half. The other vessels should then be filled,

FIG. 286.

and portions of the liver hardened and cut into sections as already described.

Fig. 286 is an illustration of an injected section of human liver.

The Spleen is injected either from the aorta, or from the splenic artery.

The Pancreas should be injected from the aorta. This organ requires to be well hardened before being cut into sections.

The Lung.—The method of injecting the lung has been already described. A double injection is made by filling the veins first and the arteries afterwards. The veins should be handled very carefully, else, on account of their delicacy, they will be easily ruptured.

The Brain and Spinal Cord are injected from the aorta. The cord requires very careful manipulation to dissect it out of the vertebral canal in such a way as not to injure it. A pair of sharp bone forceps will be found very useful for cutting through the vertebræ.

The Tongue is injected through the carotid arteries.

The Stomach is injected through the gastric artery. The veins of the stomach are to be filled by opening the portal vein, and directing the point of the syringe towards the stomach.

The Intestines.—The upper portion of the duodenum must be injected through the arteries which are derived from the cœliac axis; the lower part of the duodenum, and also the ileum, the cæcum, and the ascending and transverse colon must be injected through the arteries which are derived from the superior mesenteric artery. The descending colon, the sigmoid flexure, and the rectum are supplied with blood through arteries which originate in the inferior mesenteric artery, and must be injected through these arteries. In every case, if the vessels which pass to and from a part or an organ are large enough to admit the canula of the syringe, it is advisable to inject through them; but if a vessel will not admit the canula, inject through that vessel of which the other smaller vessel is a branch. The large vessel should, of course, be ligatured at points above and below that in which the pipe is inserted, or the injection will flow into adjacent parts.

The Lymphatics.—Ludwig's puncture method is the simplest way of injecting the lymphatics. With a scalpel,

make a slight incision in the pad of a dog's or cat's foot, and insert the nozzle of a hypodermic syringe, and inject Brücke's blue into the pad. Withdraw the syringe, close the cut with the thumb, and draw the fingers along the limb. This will force the injection through the spaces in the connective tissue into the lymphatics.

A little practice will soon enable the beginner to overcome the difficulty which attends the injecting of arteries; but it will be found that the veins, on account of their thinness and delicacy, require much more careful manipulation. A few failures may be expected at first; but after three or four trials, much of the difficulty of injecting will disappear. Careful dissection and attention to the directions here laid down will save much labour and loss of time.

CHAPTER XVIII.

THE PREPARATION AND MOUNTING OF OBJECTS.

WE have now described most of the processes incidental to microscopical research, and which should be applied to specimens in order to gain some insight into their peculiarities or their structure. No doubt many students will think that we have delayed unnecessarily the preparation of objects for mounting, and the subsequent process whereby they may become permanent objects of interest, and that we have taken pains to describe in detail processes much more difficult than mounting.

We do not think this. It may be thought an easy operation to place a piece of sea-weed in Canada balsam, and cover it with a circle of thin glass; or to soak an insect in potash, to squeeze out the internal organs, finally mounting in balsam and benzol; but this is not the style or class of mounting which we wish to see become general.

Dr. Pelletan, in a letter to the "Journal de Micrographie" (iii., No. 3, p. 139), speaks very strongly of the scientific value of microscopical preparations. In speaking of the method of mounting insects he says: "Others, more ingenious, mount large insects or immense spiders entire after having emptied them of their contents, and these preparations have really a magnificent appearance. But alas! the

integument is all that has been preserved, and the little that remains of the internal organs is represented by a uniform transparent mass, in which the microscopist finds nothing to study." He also adds further on: "I would not say that all preparations which I call trivial are useless; most certainly not. If they are not satisfactory to *savants* they interest *amateurs*, and they teach many things that otherwise would not have been known. They are also useful in England, where they are sold in large numbers, because among our neighbours the microscope is more used for amusement and as an object of luxury than for working purposes."

We cannot fully endorse the opinions of Dr. Pelletan, though it would be more satisfactory to find an improvement in the general mounting of objects; and to prepare one or two slides of a subject *well* would be of far more value than mounting a host of second-rate ones for *exchanges;* and, further, we would like to see far more experiments upon subjects mounted in different media than exists at present, and what with interchange of opinions upon this subject at our various microscopical society meetings, we could scarcely fail in rendering our cabinets more interesting and our preparations more permanent.

The mounting of objects and their preparation for this purpose is by no means an easy operation, especially if our slides are to be *permanent* and of scientific interest. The conditions are ever varying, and it requires a good knowledge of the properties of the various substances generally used in this branch in order to know beforehand what their actions will be on this or that object or portion of it. Even a process which would do well for some particular class of objects is often found to fail with some of them. If, for instance, the marine alga *Bangia fusco-purpurea* be mounted in balsam and benzol it will represent it in its natural con-

tion, while if we try to preserve *Dasya coccinea* in the same manner we shall fail miserably. In other instances we may find many algæ which may be successfully mounted in Deane's medium, while if we try to put up the first-named algæ in it we get nothing but a swollen tube containing swollen endochrome. Thus it is that many objects are spoiled by the lack of knowledge of various preparers, and also by the fact that some who should possess this knowledge perform their operations so rapidly that it is impossible they can produce uniform and permanent work. And what do we see when we cast our eyes over the contents of various cabinets? Drawer after drawer is scanned, and if the third of their contents are passable, *from a scientific point of view*, the possessor may be congratulated. What can be done to get microscopy out of this groove? Slower work, more time in preparing, more care, the rejection of all middling or bad slides, the study of the object before proceeding to mount it, mounting but few slides, and last and not least, the careful study of the effects of the various varnishes, cements, and reagents upon each other, and upon the various objects they are intended to preserve.

For the preparation and preservation of objects for the microscope certain pieces of apparatus are either necessary or useful, and although many makeshifts can be employed, we give illustrations of the various instruments most generally used.

Objects are generally mounted upon glass slides, or "slips," as they are sometimes called, which measure three inches in length by one in breadth, and of various thicknesses. They are sold either with rough edges or ground edges as may be required, but there is so little difference in price between the two varieties—the latter possessing so many advantages—that the student is strongly advised to purchase *ground edges* only.

The ordinary flatted crown slips are the cheapest, but should never be used for fine work, those of plate glass being preferable, and even this should be selected if required for mounting objects requiring delicate attention to illumination. All kinds are generally fairly clean when received from the dealer, but must, nevertheless, be beautifully polished before any object can be mounted upon them. The thorough cleansing of the glass is an important step, as the slightest film of grease is a preventive of perfect adhesion of any varnish or cement which may be subsequently used. Ammonia has the power of converting grease into soap, and spirits of wine will dissolve the two, and as some kinds of dirt require friction to remove, the following is perhaps the best formula for a mixture for cleaning slides:—

Liquid ammonia (sp. gr. ·880)	10 drops.
Methylated spirit	2 oz.
Water	½ oz.
Rouge, sufficient to make a thin cream.	

Rose-wood slips, also three inches long by one broad, are also used for mounting objects, either with sunk cells or with holes bored right through; but the student will find, perhaps only after a valuable series has been spoiled, that wooden slides possess no advantages over those of glass, and have very many objectionable qualities.

In mounting objects, a slide-centerer should be employed, in order to make the finish as presentable as possible, as it will generally be found that a slovenly finish means a bad preparation, though this is by no means *always* the case. The most simple form of centerer is shown in Fig. 287, which is simply a piece of very stiff card, upon which the lines and circles can be drawn.

Another form of centerer for slides was illustrated in "Science Gossip" for 1879, from which the accompanying

sketch is taken (Fig. 288); the device is so simple that it needs no description.

The object is nearly always covered with thin glass, either squares or circles, and is sold by the ounce in sizes for circles, increasing by eighths of an inch. It is of importance that for high powers used dry the cover-glass should be of uniform

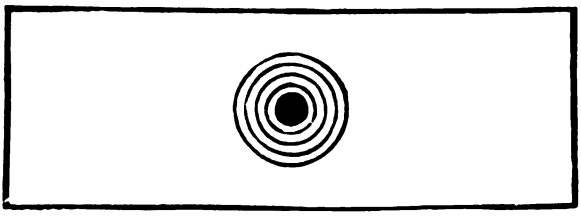


Fig. 287.

and great thinness. When purchased they should be selected according to thickness, and each variety used for its special purpose. The thickness may be ascertained in various ways. In one, the cover may be held in the stage forceps edgeways, and measured by observation with the micrometer eye-piece, or by the methods described in Chapter XIV.

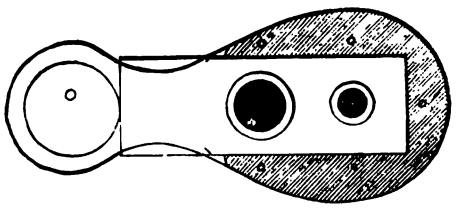


Fig. 288.

None but the very thinnest glass can be used with the dry $\frac{1}{75}$ and $\frac{1}{60}$ objectives; it is much more expensive and difficult to cut than ordinary cover-glass.

Cells and Cell-making.—Many objects requiring to be permanently mounted are of such a thickness that the thin cover needs some support at its edges, or again others are

better preserved when mounted in fluids, and for these purposes cells are employed. These are of several kinds, but may be conveniently divided into three classes, varnish cells, solid, and built-up cells.

The cell-making machine was originally devised by Mr. Shadbolt, as a simple brass circular plate about three inches in diameter, upon the surface of which was placed a pair of springs for the purpose of holding down the glass slip; it has been the parent of several forms supposed to be of great benefit to the microscopist. In order that the slides may be easily centered, the table is usually engraved with a series of circles; but it is readily seen that such centering can only be approximate.

In order to centre the slides accurately in one direction, that of width, Zentmayer introduced the simple device of fixing a couple of pins equidistant from the centre and at opposite sides of the table, the slide being so arranged that it touched both of these pins. This centres for the width, and in length this is accomplished by a series of circles near the edge of the table, the operator making the adjustment from inspection of these.

In 1870, Dr. Matthews devised a turntable to accurately centre slides in the direction of their width, and this possessed the further advantage that no springs or other portions of the table rose above the slide, to catch the fingers or brush, during its revolutions; and in the next year, Mr. J. B. Spencer, in a communication to "Science Gossip," showed how this might be made in hard wood by the microscopist himself.

In "Science Gossip" for 1874, Mr. Bridgman described and illustrated a form of turntable which, though not self-centering, enabled a slide to be always placed in the same position upon it, so far as the centre of rotation went. In 1875, Mr. C. F. Cox, of New York, devised a self-centering

turntable, consisting of the circular revolving plate, in which was cut a slot in the direction of its diameter, and in this were moved, by means of a right-handed and left-handed screw, a pair of clips which gripped the opposite and extreme corners of the slip.

It will thus be seen that so long as the edges of the slip are at right angles to each other the centering must be absolutely accurate, but not otherwise.

On the introduction of this machine several operators objected to the method of holding the slip, and Kinné soon afterwards introduced his modification, in which the two corner clips were drawn together and made to grip the glass slip by means of an indiarubber band or spiral spring.

In 1876, a notice appeared that Mr. Charles Butterworth, of Shaw, near Oldham, exhibited at the annual *soirée* of the Oldham Microscopical Society a turntable

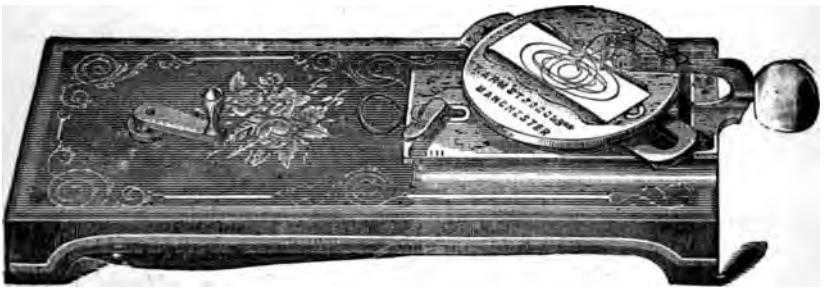


FIG. 289.

capable of making cells of either circular or elliptical form; and also by its aid a thin cover-glass could be held in position on a cell, whilst the various rings of cement or varnish could be put on. It is constructed upon the principle of the "oval chuck," and so enables either circles or ovals to be traced with ease—moreover, it may also be used for cutting thin glass covers, either oval or circular, as well as for general mounting purposes.

A turntable of exactly similar make is now sold by Messrs. Armstrong, of Manchester ; it is sold as shown in Fig. 289.

The year 1880 saw the introduction of two turntables, the first by Mr. Dunning (Fig 290), which has since been made by Mr. Swift, and the second introduced by Dr. Matthews. Mr. Dunning's turntable will take any slides up to two inches in width, and also serves for retouching slides, the circles upon which are not truly central. Dr. Matthews' last production is to be found fully described in the "Journal of the Royal Microscopical Society," and as it only consists of a method of driving the table, it will be of but little use mentioning it here in detail.

FIG. 290.

Another good form of self-centering turntable has lately been introduced by Mr. Aylward, of Manchester, and may be said the simplest, least expensive, and best self-centering turntable extant.

The author, from actual experience, can recommend it as being *the* turntable for all the wants of the microscopist. Mr. Aylward calls it the "Concentric." It essentially consists of two plates, the inner revolving on a pivot, whilst the outer revolves concentrically on the inner, a few small pins being so arranged that by a single turn of the outer ring they firmly grasp the glass slide, and cause its centre to exactly coincide with the centre of the turntable, whilst a

simple reverse movement instantly liberates it. This turntable answers for slides of various widths, from 1 inch to 2½ inches, it is strongly made, and well finished, besides which loose springs are supplied, fitting into corresponding holes in the turntable, convenient for making rings in any other position than that of the true centre of the slide.

This instrument is shown in Fig. 291, the letters being explained as follows:—

A, ordinary wood block with steel pivot, on which the brass table revolves. B, two brass springs which fit into holes in the table, and may be used when the slide is required to be out of centre; when not in use they fit into holes in the wood, as shown above. D, revolving table, with milled wheel below for rotation. H, brass annulus or ring revolving concentrically on the table D. On the ring H are screwed

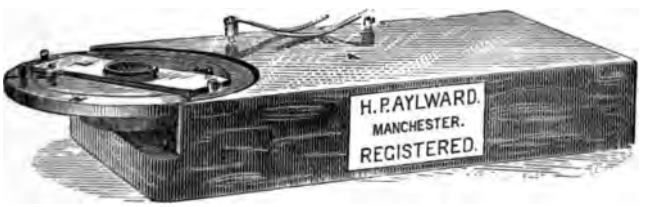


Fig. 291.

two conically headed pins, J J, $3\frac{3}{32}$ inches apart exactly, to allow the 3 × 1 slip to be placed diagonally between them. F F, two similar pins in plate D, so placed, that upon revolving the ring H they, in conjunction with the pins J J, firmly grasp the opposite corners of the glass slip, and cause the centre to coincide with the centre of the table D. I, brass pin for more easily revolving the ring H, for securing and liberating the glass slip, which is done by moving the ring H in the opposite direction.

Let us now turn our attention to the practical operation of cell-making. To commence with varnish cells—place the slide upon which the cell is to be made between the pins upon Aylward's turntable, and make three rings thereon (of the diameter the cell is to be when finished) with a writing diamond: this operation is to roughen the slip, and cause more perfect adhesion of the varnish. Now take up a good brushful of varnish, and spinning the table round, deliver it where the rings have been cut, in such a manner that it stands up like a wall, and does not spread itself more than is necessary over the slide. So soon as the layer is dry and perfectly hard, another layer may be put on, and the process repeated until the cell has acquired sufficient depth. The cell now requires drying or baking at a gentle heat over a long period, and this can be easily accomplished in the hot-air chamber shown in Fig. 292.

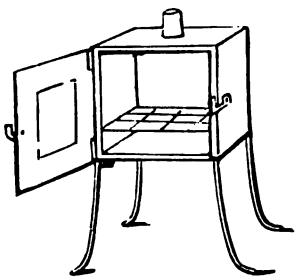


Fig. 292.

This piece of apparatus is not absolutely necessary, though it is of much assistance. By means of a spirit lamp or gas-burner almost any temperature can be maintained for lengthened periods, a thermometer inserted into the air space serving to measure the degree of heat; it serves many useful purposes, and is certainly better and more uniform than the "cool oven" so often recommended

The varnish of which a cell is constructed must vary according to the nature of the medium it is to hold; for objects mounted dry and not very thick, a brown varnish cell is all that is required (see receipt, Chapter XX.), or it may be made of asphaltum, marine glue, or gold-size, at the fancy of the operator.

The gold-size cell requires no baking, the hardening is due to an oxidation process, and wherever used it forms a most reliable cell. Varnish cells should be made some time before they are required, in order that no change takes place after the cover-glass has been put on.

Deeper cells are constructed of glass, ebonite, tin, ivory, brass, and several other substances; the glass slip forming the base of the cell should be roughened as before mentioned, the cell-ring roughened on each side, and then cemented to a glass slip with gold-size or brown varnish.

The student is advised to eschew all paper, cardboard, and wax-cells: varnish is the safest to use for thin cells, and glass, pure tin, or ebonite for the deeper ones, and if care be taken to roughen the surfaces of contact, the tendency to leak when filled with fluid will be much reduced. A considerable number of cells of various diameters and depths should be made at one sitting, and a stock of old cells always kept on hand. If new ones are used they often turn out unsatisfactorily.

Large cells may be made of four separate walls of glass all ground together to one level, cemented by their corners to the glass slide with marine glue. In using marine glue the surfaces to be united should be heated very hot, the marine glue applied to the edges, and kept firmly pressed together until set. They may then be further heated in the oven, Fig. 292, for twelve hours.

In passing now to the mounting of objects, the reader will see the impossibility of describing how any particular object or class of objects may be successfully mounted; which is the best of the several methods can only be discovered in actual practice, and if the salient points in each method of mounting be described, the student will no doubt learn much that will help him with other subjects.

For this reason, the mounting of objects has been divided into three sections :—

1. Mounting dry.
2. Mounting in gum resins.
3. Mounting in aqueous media.

DRY MOUNTING.—In treating of dry mounting we may so subdivide the work as to show the isolated operations upon which success depends, and in doing so, the student will see the importance of thoroughly understanding the why and the wherefore of each operation. These may be described as follows:—

Cleaning the Specimens.—This is a section upon which a moderate-sized volume may be written, as it applies to all objects whether mounted dry, in gum resins, or in aqueous media, and may simply be described as an operation for eliminating matter in the wrong place—dirt. Foreign matters should be eliminated as much as possible, and really, when set about in the right way, it is not very difficult. When we come to compare the slides of diatoms put up by Cole, Redfern, or Redmayne, with many home-mounted slides it may be readily seen what is the effect of a little care on the part of the preparer. Cole's exceedingly clean gatherings, his hand-picked slides, Redfern's single diatom, mounted on a $\frac{1}{4}$-inch cover in the centre of a red circle $\frac{1}{16}$ of an inch in diameter, and Redmayne's diatom slides all deserve imitation.

Let us turn again to the mounting of mosses: how many of the *οἱ πολλοί* are content with placing the specimen in glycerine jelly, dirty as when collected, making no effort at all to divest it of its useless and degrading accompaniment!

It is to be hoped that these few words will act as an incentive to cleanly working; all the requisites are several camel's-hair or sable pencils, one of which should be cut

short so that the hair projects but a quarter of an inch beyond the quill-holder. The knives, scissors, forceps, needles, and other articles have been already described in the chapter on dissections; it may, however, be necessary to state that each article should be kept for its specific uses, as if knives, scissors, and needles be used for mounting purposes they will soon be out of order for dissections.

Most objects can be cleansed under water with the brushes and needles in the small dissecting troughs shown in Fig. 208; these can be used on a watchmaker's eye-glass or on the stage of the microscope under a half-inch, with erector such as has been already described.

Before being finally finished, every slide should be examined under that power most suited to show its characteristics, and if it is defective in any way it should be discarded and washed off the slip.

The final washing of an object treated with water or any aqueous fluid should be made with distilled water—ordinary water leaves more or less residue, which interferes with the brilliancy of the preparation.

Drying the Specimens.—The moisture can be abstracted from many substances by contact with a fluid such as alcohol in a small corked tube, but in many instances this is not admissible, so that a desiccator becomes necessary. The form used by the author is shown at Fig. 293. It consists of a mahogany base-board, in which a circular groove is turned to admit the bell-jar standing over it. This groove is filled with mercury, which acts as a lute and cuts off all connection with the outer air. Under the bell-jar, and standing upon the base-board, is a vessel containing concentrated

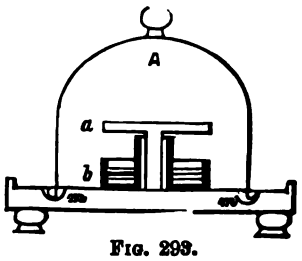


FIG. 293.

sulphuric acid, one of the most powerful absorbers of water we have. A shelf is fixed over the acid, upon which are laid the slides requiring desiccation.

Unless the operator is careful and has a safe place to keep the desiccator, he had better choose another pattern, as concentrated sulphuric acid is a fluid not to be spilled with impunity, and quicksilver is an extremely awkward metal to pick up from the floor upon which it may have been dropped accidentally. The sulphuric acid may be replaced by fused chloride of calcium, carbonate of potash, or even in quicklime, broken in pieces the size of hazel nuts; but the apparatus is not then quite so effective or rapid.

The mercury may indeed be dispensed with altogether by the use of a plate of ground-glass in lieu of the grooved baseboard, but in this case the bell-jar must have a strong and well-ground welt round its open mouth, perfect adhesion between the two being secured by means of a coating of grease.

There is a very good form of desiccator which may be obtained from almost every dealer in chemical apparatus. The lower portion contains the desiccating material, over which is placed a sheet of perforated zinc, while the cover is ground truly, and fits as a cap or lid upon the lower half. Perhaps this form would suit the majority of our readers, more especially if combined with the use of chloride of calcium as the desiccating agent.

The preparation to be dried is placed upon the shelf (Fig. 293) above the desiccating material, covered with the bell-jar, and left to itself for twelve or twenty-four hours.

In dry-mounting it is absolutely necessary to eliminate *all* the moisture, for many objects mounted in a damp state often become covered in a few months with a dense growth of fungi, or the cover-glass becomes obscured to such an extent that the object is seen as through a fog. This often happens also when a cardboard cell or wooden slide is used.

Let us now proceed to illustrate the foregoing by the process for mounting diatoms given in the "American Journal of Microscopy," April 1880.

The process of cleaning diatoms requires time, skill, patience, and personal experience, in addition to what may be learned from others. After trying for a long time to dispose of sand and mud the novice will be more careful in collecting. After an explosion or two, involving the loss of valuable material, and possibly the destruction of clothing, he will learn that strong acids and other chemicals are not to be handled like water. Experience makes the process safe and comparatively easy, requiring but a few minutes' attention at a time. No one method will apply in all cases, for some gatherings are imbedded in stone, some cemented with lime, which require special attention, while many gatherings require nothing more than a strong heat to destroy the organic matter and leave them ready for mounting.

In recent gatherings, when the diatoms are clean, put them into a bottle containing equal parts of alcohol and water, where they may be kept as long as desired. When ready to transfer them to slides, all that is required with most varieties is to dip a few from the bottle with a pipette, to put them on the thin cover-glass, and after placing the glass on a strip of mica or of tintype, keep the whole at a red heat until the organic matter is destroyed, and only the shells remain in white powder.

Another method is to boil for thirty to sixty minutes in strong soapsuds, afterwards washing thoroughly in soft water to get rid of foreign material, such as sand, flocculent matter, &c. On examination of the material, if organic matter be still present, put the mass into a test-tube or other suitable vessel, and, after settling, completely turn off all supernatant water, adding four or five times its bulk of nitric

acid, and while boiling throw in small fragments of bichromate of potash to bleach. Some prefer chlorate, but the bichromate is sufficient, and danger of explosion is avoided. When the organic matter has been destroyed, a higher temperature will be required to boil the acid, indicating that no more is needed. Probably five or ten minutes will be sufficient. Wash in rain-water or that from melted ice, until a drop evaporated on a slide shows no residue around the edge, leaving a clean slide of diatoms. Never use hard water, for the lime in it will cause all flocculent matter to cohere in masses.

The methods given are all that is required for a large proportion of diatomaceous material so far as disposing of organic matter is concerned. The sand and other indestructible matter must be eliminated by gravity.

Guano, Monterey stone, material containing lime, &c., require harsher treatment and much more time.

Guano should be boiled at least two hours in soft water, or as long as any colouring matter can be turned off; then proceed as in fossil earths.

Stone-like masses must be broken down by boiling in a strong solution of soda crystals. After disintegration, wash and boil for twenty to thirty minutes in strong nitric acid, and while yet boiling add about an equal quantity of muriatic acid, continuing the boiling for from twenty to thirty minutes longer. After washing out the acids boil in pure sulphuric acid until the mass becomes inky black, then throw in fragments of bichromate of potash, and continue the boiling until it becomes clean. If, on examination with the microscope, it is found there is much flocculent matter besides the diatoms and sand, it can be removed by boiling for a few seconds in caustic potash, and then turning *almost instantly* into plenty of soft water to destroy the action of the potash. The diatoms are now chemically free from all organic matter,

and they may be dried and kept in small phials in powder, or be put into equal parts of alcohol and water, and kept for future separation from sand and other inorganic matter, or we can proceed at once to isolate the diatoms, also to separate into sizes. To do this, put the cleaned diatoms into a small bottle, fill with soft water, filtered, and after shaking thoroughly turn off all that floats after five seconds into a larger bottle. Repeat the process, and after some five or six repetitions we shall find very little but sand in the first bottle; this we will throw away unless some very large diatoms remain, which can be removed by drying on a slide and picking with a mechanical finger. As soon as the material in the large bottle has settled, turn off the water and return the material to the small bottle and repeat the process, allowing longer time to settle. This process may be repeated five or six times, or as many times as necessary to make the separation satisfactory, allowing more time on each repetition to settle. Another excellent method is used by Christian Febiger, of Wilmington, Del., whose arranged slides have attracted much attention. Strain through No. 18 bolting cloth to obtain large diatoms. The remaining small diatoms and sand must be placed with water in a clock crystal and rotated. The sand will go to the bottom, and the diatoms can be poured off repeatedly until as clean as desired.

It will be impossible to save all the diatoms in the repeated washings. So long as 100 slides can be mounted from a mass not so large as a small pea, be content to save time and patience by losing a tithe of the harvest. Do not be disappointed when you find hardly enough diatoms remaining to make a fair thickness of carpet in your phial, for if clean you will have sufficient for yourself and for several of your friends, even then.

For mounting, always place with a pipette a drop of the

fluid containing them upon the cover-glass, and never on the slide. Professor Hamilton L. Smith is the author of the following excellent method. Cut a piece of photographer's tintype into strips about 1 inch wide and 3 inches long, then cut away all except enough for a handle, leaving 1 inch square on one end; bend the end of this handle, and fasten into a cork in a bottle, which will serve for a holder. Upon this plate place the clean cover, and by means of a pipette, drop a little of the dilute alcohol and diatoms upon it, applying a gentle heat with a spirit-lamp. The alcohol takes fire and burns off. The remaining alcohol causes the diatoms to become evenly distributed. If inclined to mat, touch with a hot pin or needle. Now bring the whole to a red heat for plenty of time to make the diatoms appear white and perfectly clean.

Now take a thin glass slip upon which a very thin cell of brown varnish has been made and well aged as previously described, take up the thin cover with diatoms attached and place it upon the slightly gummed end of a cedar-wood pencil, the diatomed side being uppermost. Now with a fine sable pencil coat the edge of the thin cover with a layer of thick brown varnish, wait until it has nearly set, and invert it carefully and firmly upon the cell-walls of the slip. A slight pressure with the pencil will cause the cover to adhere all round, when the slide may be set aside for about half an hour; it must then be placed on the turntable and a slight coat of brown varnish applied to the edge of the circle.

The slide should now be put away for a week or more, after which time it should be examined with the power suitable to it, and if not satisfactory, discarded. If, on the other hand, it is a good preparation, the student may proceed to finish it by placing it on the turntable and describing a ring of white zinc varnish so as to cover the edges of the circle, making it completely air-tight. When this is dry, various

coloured rings may be turned upon the white substratum, giving the whole a very pleasing appearance.

Some writers have deprecated this ornamentation of slides, but the author's opinion is that the time is well spent over a good slide, while a bad one, or even one of medium quality, should be washed off as soon as examined for the first time. The composition of the coloured varnishes may be found in the list of recipes at the end of this chapter.

The process of arranging diatoms is a simple one, easily done by means of a small camel's-hair pencil, with the half-inch objective. The brush should be drawn through the lips, and when using the microscope-stand shown in Fig. 19, *sitting well over it*, the side of the hand only resting on a support, it is quite easy after a little practice to remove any diatom from the field of view. For this purpose many workers use what is called a mechanical finger as a substitute for the fingers of the human hand.

FIG. 294.

Rezner's form of this instrument (Fig. 294) is much more simple than the above, and may be adapted to any microscope. It consists, as may be seen in the engraving, of a sleeve, which is passed up over the objective just far enough to possess a firm bearing, and so that the point of the bristle is in focus when depressed to nearly its full extent. In order to use this finger, the point of the bristle should be brought into the centre of the field, touching the object

slide, and then withdrawn, still in the axis of the microscope, until it has become invisible. The desired object is then sought for, such as diatoms, foraminifera, or other minute specimens, and brought into the centre of the field; the point of the bristle is then lowered by the screw until it touches the desired object, which usually adheres to it at once.

Some operators recommend a cat's whisker for the purpose of using with the mechanical finger, and no doubt it is as good as anything. Still the worker with this piece of apparatus will find that often, especially when working with ordinary bristles, it is almost impossible to deposit the object exactly where it is required, so pertinaciously does it adhere to the bristle. Professor H. L. Smith has advised the use of a slender thread of glass for this purpose, but taking all things into consideration, the cat's whisker seems to be the best.

The diatoms are picked one by one and placed where desired. To prepare the slide, put it on a turntable, and with a pen make a small circle in the centre to guide in placing. On the reverse of the slide put a tiny drop of pure distilled water, with a small fragment of gelatine in it; so that when the diatoms are arranged, breathing on them will bind them into the size. Foraminifera, polycistina, and other similar objects may be arranged in the same manner.

As another instance of dry preparation may be given a method for mounting starch granules. The starch, say from the potato, should be well washed from all foreign substances, and mixed with cold distilled water, so as to form a slightly opalescent liquid. A thin glass cover having been cleaned is breathed upon, laid level upon the table of the desiccator (Fig. 293), and a drop of the starch solution deposited upon it. When perfectly dry it may be coated at the edges with brown varnish, and inverted upon a shallow varnish cell, as

has already been described for diatoms, and finished in the same manner.

Such specimens as micro-fungi, *Penicillium glaucum* (A), *Ascophora mucedo* (B), *Papulaspora sepedonioides* (C), *Aspergillus glaucus* (D) (Fig. 295), can only be successfully mounted by the dry method, as when mounted in fluid the spores becomes detached, and the chief characteristics are lost.

The best way to mount such specimens would be to choose a cell deep enough to hold them comfortably, and place a small drop of Farrant's medium in the centre, to which is attached a portion of the substratum bearing the fungus upon it. The slide must now be placed under the

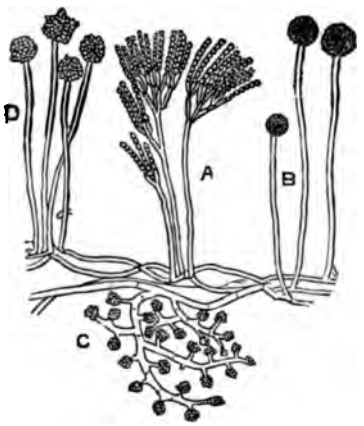


FIG. 295.

desiccator until perfectly dry, when the thin covering-glass should be placed on in the manner already described, and finished in the usual way.

There is another style of mounting it may be useful to describe, *i.e.*, in such a manner that the Lieberkuhn may be used with the slide, to produce illumination on a dark ground, so that such objects as foraminifera or the pollen of

the mallow may be viewed with this appliance, as an opaque object.

Take a very shallow varnish cell slip, but deep enough of course to contain the object, and turn upon the centre of it a disc of Bate's dead-black varnish, allow this to dry and become well aged. When proceeding to mount, say the pollen of *Althea Rosæ* (Fig. 56), dust it over this blackened disc, place in the desiccator until dry, and then put on the cover in the usual manner. If the pollen be dusted over the whole of the circle, the observer will be able to use the slide both as a transparent and opaque object. Many preparations, such as the disc of deal, do not require fixing either to the cell or its cover, and this class of objects requires no special directions for successful mounting.

MOUNTING IN GUM RESINS.—Objects required for mounting in gum resins need, of course, quite as much preparation as when put up by the dry method; but that we are about to describe is perhaps the best suited for beginners, as the gum resins are good preservatives, and the preparations are likely to be permanent with the minimum amount of care.

Canada balsam has been used for a long period for this style of mounting, either by itself or when diluted with chloroform or benzol.

We will here go through the operation of mounting a rock section in undiluted balsam, just to illustrate the only case in which the author advises the use of papers to cover up the whole of the slide.

A hot-water plate is used by a great many mounters. It may be a tin box 9 inches square by 2 inches in depth, supported on a stand or upon four legs. In one corner is a funnel-shaped neck for the introduction of water and the escape of steam when in use, the chest being heated by means of a gas-burner or spirit-lamp.

Bell-jars are sure to be required for many purposes, but principally for keeping the dust from objects which are undergoing preparation. They need not be large, and broken wine glasses may be well utilised for this purpose if the stem only is broken.

When speaking of the hot-water box, perhaps we should have mentioned the hot plate, which is still a favourite with many mounters, who still cling to undiluted balsam. This is a brass plate, standing upon four legs, and is usually heated by means of a spirit-lamp, though there is no reason why it should not be heated by gas.

The reader will remember that, when treating of section-cutting, the surface of the slice first polished was to be cemented firmly to a glass slip, the final grinding being performed upon it. When grinding a section to extreme thinness, the slip is often badly scratched, and thus disfigured. Some operators advise the section to be taken off this slip, and put upon another for permanent mounting, but in many cases this is not admissible, and the following method should be adopted. Take the glass slip with the section upon it and clean the surface with water, and after with methylated spirit; place a tiny drop of balsam (rendered fluid by heat) upon the centre of the section, carefully put on the cover, and place the whole on the hot plate, gently press down the cover, keep on the hot plate for a short time, and take away to a cool place to set.

No more balsam should be used than sufficient to reach to the edges of the glass cover, and if this point be carefully attended to, the slip will require no cleaning preparatory to covering with paper.

The covers used and recommended by Mr. I. C. Thompson, of Liverpool, may be seen as annexed.

These covers should be cut about $\frac{1}{32}$ of an inch less than the slide they are intended to cover, leaving the edges of the

ANIMAL KINGDOM	
Sub Kgd^{m}	Part .....
Class	.....
Order.....	Medium.....
Fam	.....
Gen	Mounter .....
Sp	Date.....O.G .....
.....	I. C. THOMPSON. L'POOL.

VEGETABLE KINGDOM	
Sub Kgd^{m}	Part .....
Class	.....
Order.....	Medium .....
Fam	.....
Gen	Mounter .....
Sp	Date .....O.G.....
.....	I. C. THOMPSON. L'POOL.

MINERAL KINGDOM	
.....	Object .....
Era.....	.....
Form^{n}	.....
Descript^{n}	.....
.....	.....
Locality	I. C. THOMPSON. L'POOL.
.....	

glass exposed; there is no use in binding up the glass in paper, it only helps to absorb moisture, and in the case of many objects mounted dry, serves to encourage the growth of mildew.

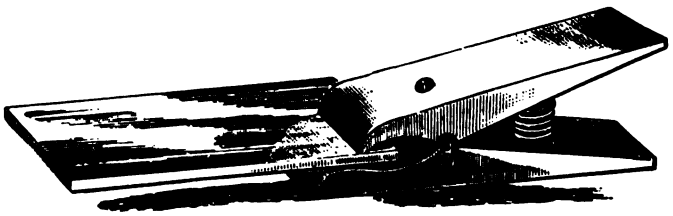


FIG. 296.

When mounting in pure balsam, especially if old, the slide gets very hot, so that the American clip shown in Fig. 296 is a convenient article.

It has already been shown, under the head of dry mount-

ing, how diatoms are distributed over the surface of a thin cover. They may also be mounted in balsam.

On the centre of the glass slip place a tiny drop of old balsam, and with a pair of tweezers place the cover-glass over it, and hold the whole over the spirit-lamp until a sea of the bubbles is seen underneath. Remove, and with a gentle pressure press down the cover. The bubbles will all disappear, and the balsam become hard. To secure the diatoms all in the same plane, turn the cover-side downwards, and leave in a warm place. This is best effected in the whalebone clip shown by Fig. 297, but care should be taken that the balsam does not project beyond the cover, or it will stick to the whalebone, and cause considerable annoyance.

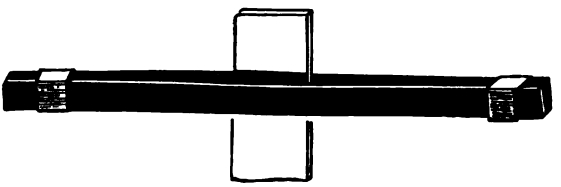


Fig. 297.

The old plan of mounting objects in pure Canada balsam has been almost abandoned; it is now usual to put up objects in balsam and benzol whenever balsam-mounted objects are required.

To illustrate how this should be done, let us proceed to mount one of the forelegs of the great water-beetle (*Dytiscus marginalis*) shown in Fig. 298.

After detaching the leg of the insect, the first operation is to soak it in potash solution for a day or two, then take it out and wash it in water, allow it to soak in dilute spirit (1 of spirit to 3 of water) for 24 hours, and then transfer it to methylated spirit. After remaining here until all the moisture has been extracted by the spirit, it must be taken out, drained on blotting paper, and placed in oil of tur-

pentine. Here it must remain until the colour of the chitinous skeleton has become sufficiently reduced, when it may be soaked for a few hours in benzol to remove the excess of turpentine. It is now ready for mounting. Place a very small drop of balsam and benzol upon the centre of a glass slip, take up the leg by means of a pair of forceps, and place it upon the drop of balsam; now put another small drop of the medium upon the object, and taking up the well-cleaned covering glass with the forceps, drop it carefully upon the object in a perfectly horizontal manner. Now *gently* press down the cover with a camel's-hair pencil in such a manner that the object is not disturbed, and put on the spring mounting clip, as shown in Fig. 299. The slide should now be heated over a spirit-lamp until the benzol *just commences* to boil, when it must be removed to a cool spot to thoroughly set.

Fig. 298.

Fig. 299.

Mr. Lofthouse, in the "Microscopical News," has given minute instructions how to proceed in mounting the proboscis of the blow-fly (Fig. 300).

Kill the fly by putting it into a bottle containing a little carbolic acid that has been rendered fluid by the addition of

a drop or two of water; no more water should be used than is necessary. Cut off the head, and place it in a small porcelain saucer, and cover with a little of the acid, which must be changed about every other day for say a week, or until it ceases to become coloured. The tongue will then, in most cases, be found protruded, or may be forced out by slightly pressing the head.

Expanding.—To expand the tongue, it should be placed in the centre of a glass slip, and put upon a piece of wood, about 5 inches long by 1¼ inch wide, into one end of which a

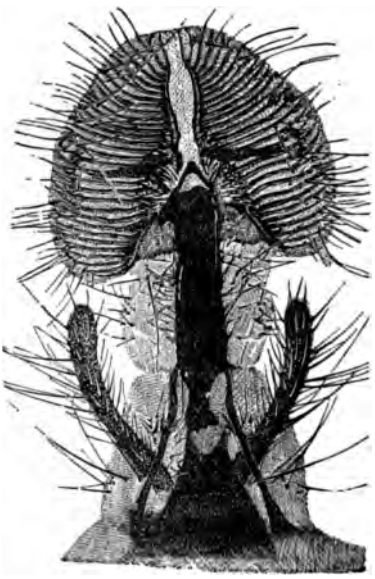


Fig. 300.

piece of wire has been inserted and bent over to form a clip, the centre being covered with a circle of *white* paper to form a light background. A piece of glass about 1 inch by 1½ inches, to be used as a presser, is placed upon the glass and under the spring, and is kept apart from the slip by several folds of paper about the thickness altogether of the fly's head. The head, with the eyes uppermost, and the tongue protruded

towards the right hand, is then placed in a drop of acid under the edge of the presser and held there, and, if necessary, the tongue forced to protrude further by a slight pressure of the forefinger of the left hand. While in this position the expander, a piece of glass 1 inch long and $\frac{3}{4}$ of an inch wide, to the under side of which a small covering glass has been fastened by brown cement, and having a piece of paper by which to hold it gummed to the top (Fig. 301), is used to force the lobes of the tongue backwards, that is towards the left hand, and downwards into the required position. The palpi, which will usually be found lying against the head, may then be arranged by means of a stiff bristle, and the head laid aside for three or four days to set.

Mounting.—After cutting away the head, transfer the tongue, which must be kept the same side up, to a drop of fresh acid on the centre of a clean glass slip. This may be done by pushing it on to the end of a quill, which has been

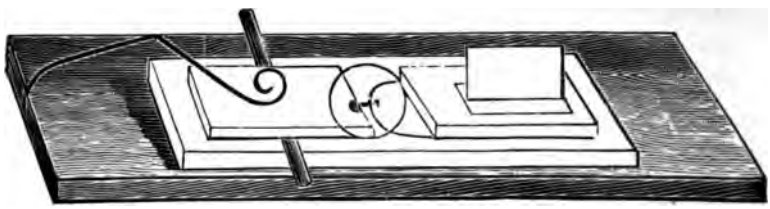


Fig. 301.

bent a little at the end, to form a kind of a spoon. Apply balsam at the right-hand side of the cover-glass, and drain off the acid by holding a piece of blotting-paper to the opposite edge. If any cloudiness appears, warm the slide a little. A light slip may then be put on, and the slide put aside to harden. No needles should be used in any part of the process.

The author's practice is, now, to set the slide on one side for a few weeks, then to clean off the excess of balsam with a scalpel, finally cleansing by slight friction with a piece of cotton wool or sponge moistened with methylated spirit

After another repose for a few days, the slide is placed on the turn-table, and a coating of brown varnish applied, so that the circle embraces the edge of the cover and the slide also, when it may be finished with the white zinc varnish and coloured rings as already described.

Balsam and benzol may be used cold, but as the operation is exactly similar to that of mounting in dammar and benzol it will not be further described.

The operation of mounting wheat-starch in dammar and benzol will sufficiently explain the cold process of mounting objects in gum resins. Make a mixture of wheat-starch and distilled water, as before advised for potato-starch; place a drop of this upon the centre of a glass cover, and put in the desiccator (Fig. 293) to thoroughly dry. Now take a drop of benzol and place upon the starch, and before it has time to thoroughly evaporate drop on a little dammar and benzol, put on the glass slip, place the spring clip upon it and set aside for several weeks, when the excess of dammar may be removed and the slide finished as already described. Where many objects are required to be mounted at once, spring clip boards may be used, but it is certainly better in many instances to have small independent clips capable of being placed in the hot chamber, air-pump, and similar situations with the slide itself. Many objects may be mounted in gum resins, and the dammar and benzol will perhaps be the best substance *generally* for objects requiring to be shown with the aid of the polariscope.

Sometimes with gum resins a source of annoyance is the appearance after a time of a white cloudiness, completely ruining many otherwise carefully mounted specimens. This is caused by dampness, or the presence of fatty matters not carefully removed before applying the balsam. If the foregoing instructions be carefully followed this mishap can scarcely happen.

Dust and dirt are the greatest enemies of the microscopist, and every operation which can be done under cover should be so arranged. In these cases very small but wide-mouthed bottles are conveniences, watch-glasses, small beakers, and stemless or footless wine-glasses will always come in useful during these operations; it should be the aim of the student to be cleanly in his manipulations, and endeavour to procure the best results with the minimum expenditure of material.

Mounting insects without pressure has been much practised of late, and the splendid preparations of Mr. F. Enock cannot be passed by unnoticed.

Few objects are more beautiful, or more instructive when properly mounted, than insects. For many years it was customary to mount such specimens under pressure, a practice which in nearly all cases resulted in the destruction of the natural relation of the various parts; but of late years, the beautiful preparations sent out by Mr. Enock and others, seem to have awakened microscopists to the desirability of mounting their specimens without pressure.

Preparation I.—Soak the specimens in liquor potassæ, until they are transparent. Wash well in distilled water, using a pipette and camel hair pencil. Transfer to 50 p.c. spirit, then to a small quantity of pure spirit, in a watch glass or soaking bottle, and allow them to stand for some hours. Then add oil of cloves, and allow the spirit to evaporate. By this method the formation of air bubbles in the interior of the specimens may generally be avoided.

Preparation II.—Wash well in distilled water. Soak in pure spirit or alcohol for some days. Transfer to carbolic acid, until sufficiently transparent. Then transfer to oil of cloves, but many mounters do not consider this necessary. This method should be used in all cases where the integument is not too opaque to allow light to pass through it before treatment; and it is especially useful in the study of the muscles.

Mounting.—Take a clean 3 × 1 slip, having a sunk cell in its centre. Just inside the edge of the cell, equi-distant from each other, cement three white glass beads, A A A, with hardened balsam. Put a small quantity of soft balsam in the centre of the cell, and gently warm it over a spirit lamp. Take the object, a wasp's or blow-fly's head, for example, and place it upon the previously warmed balsam, arranging it in the required position. Now take a clean cover glass, the diameter of which should be a little less than that of the cell, and holding it between the points of a pair of forceps, place a large drop of balsam in its centre, and allow it to fall upon the object. The edge of the cover B should rest upon the three beads. If the quantity of balsam under the cover glass is not sufficient to fill up the whole of the space between it and the slide, a little more must be allowed to run in, and

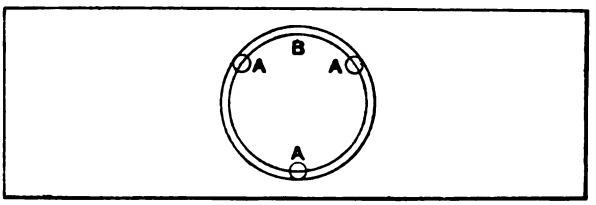


FIG. 302.

if the object has become displaced, it may be re-arranged by means of a fine blunt needle, introduced beneath the cover glass. A clip should be used during the last operations, but only to prevent displacement of the cover. The slide must now be put aside in a warm place, until the balsam is hard enough to allow the superfluous portion to be removed safely. Sufficient balsam should be left to form a sloping edge around the cover glass; and I always re-harden it for a few days after cleaning. Be sure that the balsam is quite hard before applying brown cement. The ease with which an object can be re-arranged, or a chance air bubble removed, without dis-

turbing the cover glass, constitutes the chief advantage of using beads. A supply of different sizes should be kept, and the size used must be regulated by the thickness of the object. Mr. H. C. Chadwick, from whom this method emanates, always uses pure balsam in collapsable tubes, which he strongly recommends on account of the nicety with which the quantity of balsam required for mounting a slide can be regulated. The neck of the tube should be wiped with a clean cloth moistened with benzole before the screw-cap is replaced, in order to prevent the possibility of a little balsam hardening in the screw, and so preventing the easy removal of the cap when next required.

Mounting in Aqueous Media.—This style of mounting comprises the fluids and semi-fluids, or viscid media; perhaps the latter are the easier done, but for sake of order an illustration of how to mount starches in carbolised water (*see* Recipes) will teach the method.

A suitable cell must first be selected, one of brown varnish in this instance, and filled with the carbolised water just made milky with the starch. The thin glass cover having been taken up on the end of a pencil as already described when mounting diatoms by the dry method, is edged with brown varnish, allowed a few minutes to nearly set, and then placed in contact with the cell, but not pressed down in the centre to any extent. The superfluous fluid may then be absorbed with blotting paper, the slide placed on the turn-table, and another coat of brown varnish applied to the edges. Put aside now for a day or more, and then finish with white zinc varnish or asphaltum according to the taste of the operator.

If the student possesses an air-pump he will find that fluid mounts will be rendered more permanent by placing the cell filled with fluid under the receiver so as to eliminate the air which most liquids contain. The best form of air-

pump is one with a bell receiver, but there are other forms which, though not so generally useful, are nevertheless handy on account of their small size. One of these is shown in Fig. 303, from which it will be seen that it is capable of taking slides only.

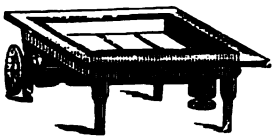


Fig. 303.

Many other fluids may be used in place of the carbolised water. The cuticle of esparto grass is shown very well when mounted in dilute acetic acid. The fibres of jute exhibit the ladder-like markings to perfection when put up in dilute spirit, while many of the desmids and minute algæ can only be kept in distilled water in which a lump of camphor is kept. Some of the smaller organisms may be put up in a

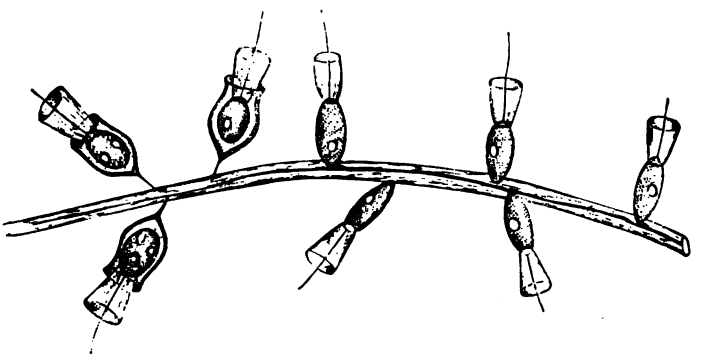


Fig. 304.

very dilute solution of osmic acid. This plan is advised by Mr. Saville Kent for the preservation of the collared monads, in his "Manual of the Infusoria," such as the *Monosiga Steinii* and *Salpingœca convallaria*, shown in Fig. 304. He states that they may be sealed up after treatment with osmic acid without the addition of any other preservative, and that it will be found the smaller and most delicate flagelliferous species are equally amenable to this treatment,

preserving their flagella, and even, in the case of the Choano-Flagellata, their sarcode collars in a life-like form. Many fluids have been described for these purposes, some will be given in the recipes at the end of the book, but the beginner should remember never to use a fluid in a cell unless he is thoroughly acquainted with the action the fluid has, or has not, upon its walls or on the cements or varnishes used in connection with it. If these points were more studied, fluid mounts would not be looked upon with so much ill-favour as they are now.

Objects mounted in glycerine should *always* be put in a cell, and if the cover be put on in the manner described for mounting in carbolised water, and diatoms, dry, the slip can be easily cleaned from any superfluous glycerine. After this,

FIG. 305.

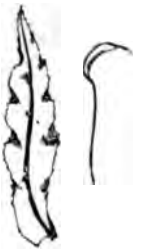


FIG. 306.

put the slide on the turntable, and describe a neat ring of brown varnish, finally finishing with a *broad* ring of white zinc varnish, as before described.

Objects for mounting in glycerine, such as a portion of muscle with Trichinæ *in situ* (Fig. 305), should be soaked for some time in a mixture of equal parts of water, alcohol, and glycerine, then exposed to the air under a bell-jar, or in the desiccator; the tissues gradually become filled with strong

glycerine, and the object is then ready for mounting in that medium.

Turning now to viscid media, the author has made use of Farrant's medium, having mounted mosses and starches very successfully in it. It is a very convenient substance, seeing that it is used cold, and the slide cleaned with water and a camel's-hair brush *immediately* after mounting; when on the following day the cover may be finished with white zinc varnish and the usual coloured rings.

The next description of mounting, that in glycerine jelly, is one which, to a beginner, is frequently a stumbling-block. It is an exceedingly convenient method to adopt when the subject would be rendered too transparent in balsam or dammar, or when it is undesirable to dry it at all.

In the preparation of the many things from the vegetable kingdom, as mosses (Fig. 306), algæ, cuticles, and sections, and from the animal kingdom, as many eyes and wings of insects, gastric teeth, palates of the Mollusca, it is only necessary, if they are sufficiently clean and not too dark in colour, to put them for a few hours into a mixture of methylated spirit, glycerine, and water (about equal parts of each), although exactness is not necessary, as the mixture may be varied to suit circumstances.

When they are taken from this mixture they must be placed upon the centre of the slide, and the surplus fluid absorbed by blotting-paper. Either of the two plans may now be followed with regard to the jelly—it may be liquefied by placing the bottle in hot water, and then dropping the liquid jelly upon the slide, or a small piece may be cut from the bottle and put upon the object and the slide gently warmed, when the jelly will diffuse itself through the object, and will be found exceptionally free from air-bubbles; but should there be air-bubbles or not, it is of great value to boil the jelly and object upon the slide, but care must be

used or the mount may be ruined. Should the boiling be decided upon, the clip should be used, and the slide held over the flame of a lamp. It will at first begin to bubble from the centre outwards, and if the slide be carefully watched, a very perceptible crack may be seen and heard. At this moment, and without delay, the slide must be withdrawn from the heat and placed upon a cold surface (an iron slab for instance), when the jelly will rapidly set and air-bubbles be excluded.

The mounts are easily cleaned from superfluous jelly, by brushing with a *soft* tooth-brush under a running water tap. the surface of the slide being allowed to dry spontaneously. It will be found that the slide is free from glycerine smears, which interfere much with the after-process of finishing.

The slides may then be finished by ringing with white zinc varnish and the various coloured circles.

FIG. 307.

Such is the method employed for mounting in glycerine jelly, and very satisfactorily illustrates the general practice, though, of course, it is not all objects which will bear boiling.

An instrument, called Smith's mounting machine, and shown in Fig. 307, is very useful when mounting large and elastic substances in glycerine jelly, as by it any degree of pressure may be exerted upon the covering glass.

The method of using this instrument may be explained by mounting a lichen section (Fig. 308) in Deane's medium.

The section having been cut as thinly as possible, it is to be soaked for a day or more in some of the medium, diluted with just enough water to render it fluid; it is then to be placed upon a slip, the cover superposed and placed in the mounting instrument, a gentle pressure being exerted by means of the screw. The slide must now be warmed over the lamp, and a drop of the medium placed at the edge of the cover, when it will be drawn under by capillary attraction. This is a very clean way of mounting specimens, and one often used by the author.

FIG. 308.

FIG. 309.

The foregoing instructions have been given more as an aid to the student than to the expert mounter. It would be impossible to give processes for mounting everything, as even the preparation varies in nearly every specimen. All that has been said to enable the beginner to practise the art of mounting has been written in a general sense, and each process must be carried out *with intelligence*. The student should endeavour to make his preparations look as natural as possible, and no pains should be spared in this respect. Again, the question of varnishes and cements is one which demands careful study, and the beginner is advised to well peruse Chapter XX. before he commences actual work.

When the slides are mounted, a cabinet is required to

keep them in. They are generally made to one pattern, and may be obtained from any dealer in microscopical apparatus. A special form of slide box was devised some time ago by Messrs. Jas. Queen and Co. of Philadelphia. It is shown in Fig. 309, and it certainly has compactness and portability to recommend it.

We cannot close this chapter without exhorting every microscopist to keep a catalogue of his slides and a minute description of each one, setting forth the points of interest to be observed. In this connection Maltwood's finder, shown in Fig. 310, will be found most useful.

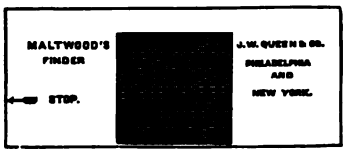


FIG. 310.

Maltwood's finder consists of a photograph of a series of numbered squares, all the lower figures in each horizontal row, and also the upper numbers in every vertical column being similar, as shown in the accompanying diagram :—

30 23	31 23	32 23	33 23
30 24	31 24	32 24	33 24
30 25	31 25	32 25	33 25
30 26	31 26	32 26	33 26

A stop should be attached to the stage, so that the slide and finder may always be placed in the same position.

To use the finder, lay the object slide on the stage and bring the point to be recorded exactly in the centre of the field; now put on the finder in place of the object (taking care not to move the stage) and record the numbers of the square in the centre of the field. When the object is again desired, place the finder against the stop on the stage, and bring the recorded numbers into the centre of the field. When the finder is replaced by the slide, the desired point should at once be visible.

Maltwood's finders should agree with each other, no matter wherever purchased. But unfortunately they do not always do so, and so observers at a distance from each other are placed under disadvantages which have no right to exist.

CHAPTER XIX.

REAGENTS.

LITTLE now remains but to notice the action and properties of some of the various reagents used for microscopical purposes, and in order to put them in something like order they are divided into four classes—softening agents, dehydrating and hardening agents, bleaching and oxidising agents, and solvents.

Softening Agents.—One of the mildest softening agents with which the microscopist has to deal is acetic acid diluted with four times its weight of water; it renders some tissues quite transparent, and used in conjunction with glycerine serves for sending insects for dissection to long distances. Acetic acid dissolves phosphate and carbonate of lime, but does not dissolve oxalate of lime, and neither has it any action in the diluted state upon any of the varnishes or cements in the general use of the microscopist.

Glycerine is a fluid of the greatest use in microscopical research, particularly as an agent for preventing the drying up of tissues. One part of pure glycerine diluted with nine parts of pure water produces a fluid of the same density as sea-water, and this strength is a very handy one to use. Contrary to the general opinion, cells filled with glycerine do *not* necessarily leak, it is only when objects have been *slovenly* mounted in this medium that such a mishap occurs.

The alkalies, potash, soda, and ammonia are somewhat

alike in their action upon many substances, ammonia being the weakest reagent. When used in concentrated solution, animal substances, especially of an albuminoid nature, are entirely dissolved, while, when used in a weaker form, many organs are separated in such a way as to be readily obtained. Upon vegetable substances the alkalies act somewhat similarly, the leaf of the esparto grass (*Macrochloa tenacissima*) and the straw of cereals, and even blocks of pine wood, are all softened and disintegrated by boiling in a solution of caustic soda containing 50 grains to the ounce of water. Dilute nitric acid (one part of acid to ten of water) may also be used as a softening agent in certain cases, and, it may be added, should be used in glass or porcelain vessels, as metals are rapidly dissolved by this acid. This remark applies also, though in a lesser degree, to acetic acid.

Dehydrating and Hardening Agents.—Dehydrating agents for use with the desiccator (Fig. 293) are not put in contact with the preparation; but placed below in a separate compartment underneath a bell-jar, as shown in the figure. Anhydrous phosphoric acid is, perhaps, the most powerful absorbent we have, and next to this in usefulness is concentrated oil of vitriol.

Fluids, however, are not so handy as solids, and therefore for general work *fused* chloride of calcium, quicklime, or carbonate of potash are more generally used.

When, however, a substance requires to be dried or dehydrated without losing its fresh or moist appearance, it must be brought in contact with a water-absorber, such as alcohol. Absolute alcohol is an excellent fluid for this purpose, and is not equalled by any other reagent; methylated spirit is often used in its place, and for many purposes will suit admirably.

It must not be forgotten that alcohol has a more or less solvent action upon most of the gums used for microscopical

varnish making, and therefore strong alcohol cannot be used in such cells. Weak spirit has, however, no action upon a cell of great size, and this is the only varnish the author advises to be used when an object is mounted in weak spirit.

As to hardening agents, the chromates have been treated of in Chapter X., on section-cutting, and therefore it is not necessary to recapitulate their uses.

Tannin dissolved either in water or alcohol is a hardening agent especially useful for gelatinous tissues: it is used sometimes in injecting for hardening the walls of blood-vessels to prevent the passage of the coloured gelatine through them. Alcohol itself is a very powerful but safe hardening agent, and when combined with tannin may find many uses.

Perchloride of mercury solution has been recommended as a hardening agent, and it probably acts by forming insoluble compounds with the albuminoid matters. It is so deadly a poison that the author can scarcely advise the student to use it.

Picric acid, easily purchased in a one per cent. solution, is also a splendid hardening agent, and is of great use in studying the lower forms with naked protoplasm. It is, however, very poisonous. By the action of this reagent the currents in the protoplasm of Myxomycetes are instantly suspended, and in a few moments the plasmodium is sufficiently hardened to make sections possible.

Bleaching and Oxidising Agents.—Under this class we have:—

Chlorinated soda (as Labarraque),
Chloride of lime (bleaching powder),
Chlorate of potash,
Bichromate of potash,
Nitric acid,
Turpentine,

all of which have their special uses.

The first two are principally used for bleaching vegetable sections, and the strength of the solution may be found in the next chapter. It is most important, when using these reagents, to eliminate every trace of them after the operation is finished, and this may be done by soaking in a bath of neutral sulphite of soda, 15 grains to the ounce of water; a solution of one part of strong liquid ammonia to twenty parts of water will also effect this.

Chlorate of potash is generally used for cleaning diatoms, in connection with some strong acid, such as sulphuric, nitric, or hydrochloric. Great care should be used in making these mixtures, as it is easy with them to produce explosions, and their use should (in connection with chlorate of potash) be avoided as much as possible. Nitric acid by itself is a powerful bleacher and oxidiser, but has the disadvantage of emitting powerfully noxious and acid fumes; when slightly diluted to prevent this, its oxidising powers can be increased by the addition of chromic acid or bichromate of potash. This admixture is not in the least likely to explode.

Another extremely powerful oxidiser is a mixture of strong sulphuric acid and chromic acid in crystals; it is especially useful in cleansing diatoms, and must be heated carefully over the spirit-lamp to obtain its maximum effect. Peroxide of hydrogen is an extremely valuable bleaching agent and oxidiser, and likely to come into general use when its properties are better known to microscopists; it is neither acid nor alkaline, and does not give off any objectionable odour. Another bleaching agent is turpentine, almost exclusively used to reduce the intensity of colour in the chitinous skeletons of insects. After having been treated with potash or soda solution, and the abdominal contents expressed, the insect is washed with water, dried in alcohol, and allowed to soak in turpentine until the colour of the chitin is sufficiently reduced in intensity. Turpentine has

a solvent action upon many of the varnishes used for microscopical purposes, and therefore great caution should be exercised in this respect.

Solvents.—Under this heading come many reagents of diverse character: we have alkalis, acids, and neutral compounds, all of which have their special applications. Amongst the first class are solutions of potash, soda, and ammonia, having a great affinity for most animal substances. Grease unites with any of these, producing a soap which easily dissolves in water. The alkalis in their solid state should be handled with caution, owing to their corrosive nature. They are chiefly used in solution to dissolve out the internal organs of insects, and to prepare the leaves of mosses for exhaustive scientific examination.

The acids, sulphuric, nitric, hydrochloric, and acetic, have each their uses; for dissolving metals, oxides, carbonates, phosphates, and other salts, they are necessary. They have no action upon silica, but upon animal and vegetable fibres the action is very decided.

By far the most important solvents we have to consider are ether, alcohol, benzol, glycerine, oil of cloves, oil of cajeput, and last, and not least, water. Ether is not very soluble in water; it dissolves in about fourteen parts at the ordinary temperature of the air; it carries into solution many organic compounds, as the volatile oils, resins, fats, alcohols, tannic acid, which are but sparingly soluble in alcohol, while it is without action upon many substances easily dissolved by that reagent. It is miscible in all proportions with alcohol, bisulphide of carbon, and naphtha.

Alcohol is probably the most used fluid in microscopy; it is miscible in all proportions with wood-spirit, chloroform, acetic acid, and naphtha, and is a good solvent for most resinous substances. Attention should be paid to the strength of the alcohol; alcohol absolute is expensive,

and though a good drier, sometimes fails in its action because it is too strong. Ordinary druggists' alcohol of 85 per cent. is an excellent solvent for resins, camphor, tannin, the balsams, iodine, acetic acid, and castor oil, but the ordinary "spirits of wine," consisting of equal volumes of druggists' alcohol and water, has not the same solvent action for resinous matters. Methylated spirit consists of ordinary alcohol, mixed with 10 per cent. of wood-spirit, and is capable of being put to the use of ordinary alcohol by the microscopist.

Benzol is a colourless, strongly refracting liquid, of a very inflammable nature. It is almost insoluble in water, but dissolves freely in alcohol and ether. It dissolves iodine, sulphur, fats, gum-resins, and many other compounds, such as caoutchouc and gutta percha.

Petroleum naptha, called also benzoline, benzine, gasoline, &c., is nearly as useful as coal-tar benzol for the purposes of the microscopist, provided it be of good quality and does not contain any quantity of heavy oils.

Glycerine is a solvent strongly recommended for general use by Dr. Beale. It is soluble in all proportions in water and alcohol, though it is but sparingly soluble in ether. It dissolves nearly all organic substances soluble in water, and many of those soluble in alcohol; it dissolves small quantities of carbonate of lime and many oxides; 20 per cent. of arsenious acid, and 10 per cent. of tannic acid, forming with this latter a waxy solid, melting at the temperature of the body, which the author has used sometimes for mounting purposes.

Oil of cloves is often used as an intermediate bath between alcohol and Canada balsam; it serves to take out the alcohol, with which the oil changes place. One volume of oil of cloves dissolves an equal volume of alcohol.

Oil of cajeput is often recommended for use in place of

oil of cloves, and is in every way cheaper, as one volume of it absorbs eleven volumes of alcohol, or is eleven times more efficient than oil of cloves.

Water is the last solvent we have to consider. A great many substances are soluble in this menstruum, and even those generally considered insoluble are acted upon to such an extent as, in many cases, to interfere with its use for microscopical purposes. The component parts of the atmosphere, nitrogen and oxygen, are, to a certain extent, soluble in water, and often cause the microscopist no little trouble; and, further, it should be stated, that when water is to be evaporated on a slide for future mounting, distilled water only should be used, so that no objectionable residue is left.

CHAPTER XX.

RECIPES.

THE following formulæ are given in order that microscopists may, when desirable, be able to compound them for themselves. The author is of the opinion, however, that it will be found cheaper, where only small quantities are required, to purchase from a dealer in microscopical sundries, as most of these articles can only be *satisfactorily* made in large quantities. On the other hand, he would strongly advise that the dealers in "secret nostrums" be not encouraged; the microscopist who values the excellence and permanence of his preparations has a right to know what he is using in such an important operation as the preparation and mounting of objects.

Carbolic Acid (*Fluid*).

Procure an ounce bottle of Calvert's pure crystallised carbolic acid, place it in a jar of warm water to melt; then add two drachms of methylated spirit, mix, and preserve for use.

If strong carbolic acid be spilled upon the hands, it must be *immediately* wiped off with *oil*, not water.

Carbolic Acid Water.

Strong.

Fluid carbolic acid	1 drachm.
Distilled water	16 oz.

Weak.

Carbolic acid water (strong)	1 oz.
Distilled water	9 oz.

CHLORINATED SODA (*solution*).

Dry chloride of lime	2 oz.
Soda crystals (washing soda)	3 oz.
Water	2 pints.

Mix the chloride of lime with half the water, and dissolve the soda i the other half, mix the whole together, and allow to settle in a well-corke bottle. Pour off the clear liquid for use, which must be kept in a wel corked bottle.

CHLORINATED LIME (*solution*).

Dry chloride of lime	½ oz.
Water	1 quart.

Dissolve, allow to settle, and use the clear solution

LIQUOR POTASSÆ (*solution*).

Caustic potash (*in sticks*)	1 oz.
Water	1 quart.

Caustic soda may be substituted for the potash for nearly all purposes.

IODINE SOLUTION.

Iodine	40 grains.
Iodide of potassium	60 grains.
Water	1 pint.

Dissolve the two substances in four ounces of the water, then add th remainder of the water.

GUM WATER.

Gum arabic	4 oz.
Glycerine	¼ oz.
Weak carbolic acid water	4 oz.

Allow to stand in the cold until dissolved.

This will be found an excellent medium for attaching lables to glass.

SPICER'S FLUID.

Alcohol	3 fluid oz.
Distilled water	2 fluid oz.
Glycerine	1 fluid oz.

GOADBY'S FLUID, No. 1.

Bay salt	4 oz.
Alum	2 oz.
Corrosive sublimate *	4 grains.
Water	2 quarts.

Dissolve the salts in water, and filter, when it will be ready for use.

GOADBY'S FLUID, No. 2.

Bay salt	8 oz.
Corrosive sublimate *	2 grains.
Water	1 quart.

* The corrosive sublimate is exceedingly poisonous, so that great care must be taken in its use.

Camphor Water.

Distilled water	1 quart.
Tincture of camphor	1 drachm.

Well mix, but use only the clear fluid.

Thwaites' Fluid.

Rectified spirit	1 oz.
Creosote (wood)	q. s.
Distilled water	1 pint.
Precipitated chalk	q. s.

Saturate the spirit with creosote, then add the water and a little precipitated chalk. Filter, when the liquid is ready for use.

Ralf's Liquid.

Bay salt	10 grains.
Alum	10 grains.
Distilled water	10 oz.

Glycerine and Acetic Acid.

Glycerine	2 oz.
Glacial acetic acid	½ oz.

Glycerine and Gum.

(*Farrant's Medium.*)

Gum arabic (*best picked*)	4 oz.
Distilled water	4 oz.
Glycerine	2 oz.

Dissolve in the cold.

Deane's Medium.

Nelson's gelatine	1 oz.
Honey	5 oz.
Creosote (wood)	6 drops.
Alcohol	½ oz.
Water	5 oz.

Soak the gelatine in 4 oz. of the water for twelve hours, add the honey, previously heated to nearly boiling point, in a separate vessel, and boil the whole together. When cooled somewhat, add the spirit and water in which the creosote has been dissolved. Afterwards, filter through fine flannel.

Glycerine Jelly (*Lawrance's*).

Gelatine	1 oz.
Glycerine	6 drachms.
Camphorated spirit of wine	¼ oz.

Cover the gelatine with cold water, and allow it to soak until it becomes soft. Dissolve by placing the jar containing it in a vessel of boiling water, and allow it to cool, then add a small quantity of the white of an egg, and boil the mixture until the albumen coagulates, when the whole is to be filtered through fine flannel, and mixed with the glycerine and spirit.

Liquid Glue.

Dissolve shellac in wood-naphtha at a very low temperature, until the mixture is of the required consistency.

It makes a very brittle varnish, but very good cells.

BROWN VARNISH.

Pure indiarubber	20 grains.
Bisulphide of carbon	q. s.
Shellac	2 oz.
Methylated spirit	8 oz.

Dissolve the indiarubber in the smallest possible quantity of bisulphide of carbon, and add this to the alcohol in such a manner that the whole is mixed without the formation of clots. Now add the shellac, and place the jar containing the mixture in boiling water, until the whole of the shellac is dissolved, and the smell of the bisulphide has disappeared.

GUAIACUM VARNISH.

Gum guaiacum	2 oz.
Shellac	2 oz.
Methylated spirit	10 oz.

Powder the guaiacum and dissolve it in the spirit, filter, and then add the shellac. Keep the whole in a jar surrounded by warm water until dissolved.

MATT VARNISH.

Gum mastic	40 grains.
Gum sandarac	160 grains.
Methylated spirit	4 oz.
Benzol	1½ oz.

BLACK MATT VARNISH.

Gum mastic	50 grains.
Gum sandarac	200 grains.
Methylated spirit	1½ oz.
Benzol	½ oz.

Dissolve the gums in the fluids and triturate in a mortar with sufficient lamp-black of the finest quality.

MARINE GLUE.

Indiarubber shreds	2 oz.
Shellac	2 oz.

Dissolve the indiarubber in solvent mineral naphtha, add the shellac in powder, and heat until liquefied, well mixing the whole together. It produces a solid marine glue, and requires heat in its application. The author has it on good authority that very little of the marine glue at present made contains a particle of indiarubber.

PHOTOGRAPHIC VARNISH (*for negatives*).

Gum benzoin	¼ oz.
Gum sandarac	1 oz.
Methylated spirit	12 oz.

FRENCH POLISH.

Shellac	3 oz.
Gum sandarac	½ oz.
Methylated spirit	1 pint.

French polish is sometimes coloured with gum dragon, &c.

Brunswick Black or Black Japan.

Egyptian asphaltum	4 oz.
Linseed oil	4 oz.

Boil together for some time, and mix to the required consistency with oil of turpentine.

Canada Balsam Varnish

(*for rendering ground glass transparent*).

Take 4 oz. of Canada balsam, and bake in a cool oven till quite brittle when cooled. Dissolve this in 12 oz. of benzol, in which ½ oz. of mastic has been previously dissolved.

Black Varnish (*Davies*).

Indiarubber shreds	30 grains.
Egyptian asphaltum	4 oz.
Solvent naphtha (mineral)	10 oz.

Dissolve the indiarubber in the naphtha, add the asphaltum, using heat if necessary.

Balsam and Benzol.

Bake the Canada balsam in a cool oven, and then dissolve to the right consistency with benzol. If baked too long the residue will be too brittle; if too short, it will not dry quickly enough.

Dammar and Benzol.

Gum dammar	1 oz.
Benzol	2 oz.

Mastic and Benzol.

Gum mastic	4 oz.
Benzol	3 oz.

Gold Size.

Linseed oil	25 oz.
Red-lead	1 oz.
Powdered white-lead	q. s.
Yellow ochre	

Boil the oil and red-lead together for about three hours, taking care it does not burn or boil over; pour off the clear fluid, and boil again with a mixture of equal parts of the white-lead and yellow ochre, added in small successive portions. Pour off the clear fluid for use.

Black Gold Size.

Triturate in a mortar 1 fluid oz. of gold size with sufficient lampblack to form a dense block colour. If too thick, it may be thinned with a little turpentine.

Finishing Varnishes

(for white and coloured rings).

These are made by triturating the various colours with the vehicle in a mortar. It will be found best, after preparing each colour, to place them in saucers such as are used by artists: the benzol then evaporates, and the colour can be taken up in a brush, as required, by simply moistening it with benzol.

The vehicle:—

Gum dammar	3 oz.
Gum mastic	1 oz.
Benzol	6 oz.

The colours:—

White	oxide of zinc.
Blue	ultramarine.
Red	carmine.
Black	lampblack.
Green	verdigris.
Yellow	chrome-yellow.

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